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Begin the article with a short summary.

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Submit each table on a separate page, typed double spaced. Please use standard abbreviations; otherwise spell out.

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Medical Oncology Practice Study

The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1976.

Evaluation of an Intermittent Schedule of Dibromodulcitol in Breast Cancer[1,2]

D. C. Tormey,[3,4,*] G. Falkson,[5] E. Perlin,[6] J. Bull,[3] J. Blom,[7] and M. E. Lippman[3]

SUMMARY

Dibromodulcitol was administered orally on Days 1–10 every 3–4 weeks to 29 patients with metastatic breast carcinoma refractory to previous combination chemotherapy. Initial doses between 70 and 280 mg/m²/day were utilized. The dose was escalated as tolerated in subsequent cycles in individual patients. Hematosuppression was dose-limiting. At doses of < 200 mg/m²/day leukopenia (< 1000 cells/mm³) and thrombocytopenia (< 25,000 platelets/mm³) occurred in one of 28 cycles and two of 27 cycles respectively. In contrast, at doses of ≥ 200 mg/m²/day leukopenia and thrombocytopenia occurred in four of 18 cycles and five of 18 cycles respectively. Recovery of leukocytes (> 4000 cells/mm³) and platelets (> 100,000 platelets/mm³) by Day 29 after the start of therapy was also delayed at higher doses. Responses were observed in three of 29 evaluable patients and subjective improvement of osseous disease in one additional patient. A dose of 180 mg/m²/day × 10 every 28 days is recommended in previously treated patients to avoid severe hematologic side effects.

[Cancer Treat Rep 60:1593–1596, 1976]

Dibromodulcitol (Dbd) is a halogenated hexitol whose primary mechanism of action appears to be alkylation (1,2). Initial clinical trials led to the establishment of a dose of 3.5 mg/kg/day (3–5). Responses were observed in eight of 20 patients with breast cancer in these early trials (3,4). The drug was tested at the 3.5-mg/kg/day dose schedule in a broad phase II study by the Central Oncology Group (COG) (6). Responses were reported in 13 of 33 patients with metastatic breast carcinoma. The predominant side effects observed were hematosuppression, nausea and emesis, and reversible hepatic dysfunction.

Our interest in the drug was stimulated by the observation that some of the responses observed in the prior trials occurred in patients resistant to other alkylating drugs as well as its structural dissimilarity from cyclophosphamide, oral formulation, and low incidence of nonhematologic side effects. In addition, the capability to achieve cerebrospinal fluid (CSF) levels of about 50% of that of plasma (2) also suggested that the agent might be a useful alkylator in settings involving central nervous system metastases. These considerations led us to conduct a phase I trial to obtain an estimate of a tolerable intermittent dose schedule for subsequent integration into combination-chemotherapy regimens.

MATERIALS AND METHODS

Twenty-nine women with progressive, biopsy-proven, disseminated breast carcinoma were entered in the study. Other eligibility criteria included failure on prior chemotherapy, a Karnofsky performance score (PS) > 30%, no second cancer

[1]Received June 22, 1976; revised Sept 27, 1976; accepted Oct 7, 1976.

[2]Dibromodulcitol: NSC-104800; CAS reg. No. 10318-26-0; galactitol, 1,6-dibromo-1,6-dideoxy-.

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[7]Oncology Section, Hematology-Oncology Clinic, Walter Reed General Hospital, Washington, DC.

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other than breast cancer, no major organ disease unrelated to the tumor, and an appropriate informed consent form.

Dbd was administered orally on Days 1–10 of each 21–28-day cycle of therapy. The initial dose was 70 mg/m²/day. Dose increases in subsequent and individual patients were allowed as dictated by patient tolerance. Therapy was continued in each patient until the development of further progressive disease (PD) or relapse (vide infra).

A CR was defined as the disappearance of all clinical and laboratory evidence of disease, including normalization of osseous roentgenograms, and a return to a 100% Karnofsky PS with no disease-related analgesia requirements. A PR was defined as a ≥ 50% reduction in the sum of the products of the longest perpendicular cross-sectional measurements of the lesions in the involved organ system. In situations where lesions were only measurable unidirectionally, eg, liver enlargement, a 30% reduction was required, and in evaluable but nonmeasurable situations, eg, lymphangitic pulmonary metastases, an estimated 50% reduction in disease was required. In osseous metastases partial recalcification was required. Mixed osteoblastic and osteolytic lesions were not considered evaluable. Hepatic disease was usually evaluated as the sum of the measurements in the right and left midclavicular and xiphoid lines. The reduction in tumor had to be present in ≥ 50% of the involved organ sites for at least two measurement periods separated by ≥ 2 weeks to qualify as a PR. Improvement (IMP) was defined only for osseous disease as a major decrease in pain medication or a 20-point improvement in the Karnofsky PS if those changes subsequently persisted for at least 8 weeks and were not accompanied by roentgen changes associated with a response or PD. A no change (NC) status was designated in those situations where criteria did not yet exist for a response, IMP, or PD after 8 weeks of therapy. PD was defined as the appearance of any new lesion or a > 25% increase in any existing lesion. Relapse was defined as PD occurring after attainment of a response or IMP, or an NC status for > 8 weeks; the baseline measurements utilized for comparison were the minimal measurements obtained during the response, IMP, or NC status.

Hematologic nadir toxicities were defined on a 0–4 scale as follows:

0 = wbc count (≥ 4000 cells/mm³), platelet count (≥ 100,000 platelets/mm³), fall in hemoglobin level (≤ 1 g/100 ml);

1 = wbc (3000–3999), platelets (75,000–99,999), fall in hemoglobin (1.1–2.0);

2 = wbc (2000–2999), platelets (50,000–74,999), fall in hemoglobin (2.1–3.0);

3 = wbc (1000–1999), platelets (25,000–49,999), fall in hemoglobin (3.1–4.0); and

4 = wbc (< 1000), platelets (< 25,000), fall in hemoglobin (> 4.0).

RESULTS

Toxic Effects

The dose-limiting toxic effects were hematologic and are detailed in tables 1 and 2 for the evaluable cycles of therapy. At a dose of < 200 mg/m²/day severe leukopenia (< 1000 cells/mm³) occurred in one of 28 cycles (3.6%) and thrombocytopenia (< 25,000 platelets/mm³) occurred in two of 27 cycles (7.4%). In contrast, at doses ≥ 200 mg/m²/day severe leukopenia and thrombocytopenia occurred in four of 18 cycles (22.2%) and five of 18 cycles (27.8%) respectively. The median nadir day of leukocyte suppression increased from Day 15 (< 170 mg/m²/day) to Day 22 (> 230 mg/m²/day), whereas the median nadir of platelet suppression increased from Day 18 (< 170 mg/m²/day) to Day 27 (> 230 mg/m²/day). The mean time spent at the wbc count < 3000 cells/mm³ with Dbd doses of < 170, 170–191, 200–221, and > 230 mg/m²/day was 0, 2, 5.5, and 10+ days respectively; the corresponding mean time spent at the platelet level < 75,000 platelets/mm³ was 3.7, 0.3, 0.4, and 7.4 days respectively. At doses of > 200 mg/m² wbc counts < 1000 cells/mm³ or platelet counts < 25,000 platelets/mm³ persisted for a mean of 0.7 and 1.9 days (ranges, 0–8 and 0–9 days) respectively. Recovery of the wbc count by Day 29 to enable 100% drug dosage in the subsequent cycle decreased from 12 of 14 cycles (86%) at doses < 170 mg/m²/day to six of nine cycles (67%) at doses > 230 mg/m²/day. Similarly, the platelet count recovery by Day 29 decreased from 11 of 14 cycles (79%) at doses < 170 mg/m²/day to five of nine cycles (56%) at doses > 230 mg/m²/day. Grade 2 or 3 hemoglobin decreases occurred in 11 of 48 cycles of therapy and did not appear to be dose related. A grade 4 hemoglobin decrease occurred during one cycle.

Nonhematologic toxic effects did not appear to be dose related and consisted of the following: alopecia (one patient), local infection (one patient), sepsis (two patients), lethargy (one patient), transient SGOT rise from 47 to 157 IU (one patient), dysuria (one patient), nausea (three patients), and nausea and emesis (three patients).

Therapeutic Effects

No patients were disqualified from the analysis. There were three PRs (210, 233, and 280 mg/m²), one IMP (210 mg/m²), four NCs, and 21 PDs. The time

TABLE 1.—Relationship between Dbd dose and wbc toxicity

Dbd dose	Total No. of cycles	Nadir wbc toxicity grade—*				Median day of nadir (range, No. of cycles)	No. receiving 100% dose on Day 29/total evaluable cycles
		0	1–2	3	4		
< 170	17	10	5	1	1	15 (8–57, 14)	12/14
170–191	11	5	6	0	0	16 (15–31, 8)	7/10
200–221	9	2	5	1	1	21 (8–26, 9)	7/9
> 230	9	1	4	1	3	22 (14–43, 7)	6/9

*See Materials and Methods for definition of toxicity grades.

TABLE 2.—Relationship between Dbd dose and platelet toxicity

Dbd dose	Total No. of cycles	Nadir platelet toxicity grade—*				Median day of nadir (range, No. of cycles)	No. receiving 100% dose on Day 29/total evaluable cycles
		0	1–2	3	4		
< 170	17	10	3	2	2	18 (8–35, 15)	11/14
170–191	10	7	2	1	0	17 (8–31, 9)	8/10
200–221	9	2	4	1	2	22 (21–29, 9)	6/9
> 230	9	4	2	0	3	27 (14–35, 7)	5/9

*See Materials and Methods for definition of toxicity grades.

from initiation of therapy to response was 7 days for the patient with IMP and 28, 28, and 63 days for those with a PR. The median time from start of therapy to therapeutic failure was 112+ days (range, 52–119+ days) for patients with a PR plus IMP status, 63+ days (range, 21+–105 days) for patients with an NC status, and 24 days (range, 10–40 days) for patients with a PD status.

The median age of the patients was 51 years (range, 30–67 years). Eighteen of the 25 postmenopausal patients had had a previous oophorectomy. The menopausal status of three patients was uncertain. The dominant disease was visceral in 16 patients (two responders), osseous in eight (one IMP), and soft tissue in five (one responder). The median number of organ sites that were involved was three and the median Karnofsky PS was 70%. The responding patients had one, two, three, and three involved organ sites with PSs of 40%, 70%, 70%, and 80% respectively. Each of the responders had previously been treated and failed combination-therapy regimens with cyclophosphamide; two had had a previous response to such therapy. One of the patients had also failed to respond to a thioTEPA and vincristine combination and another patient failed to respond to a melphalan, adriamycin, and vincristine regimen. The patients had been previously treated with an average of 1.7 chemotherapy regimens.

DISCUSSION

Dbd was chosen for further investigation because of its potential non-cross-resistance with the commonly used agent cyclophosphamide, its known penetration into the CSF (2), and the encouraging results reported by other investigators (3,4). Recent preliminary results also suggest that Dbd may be non-cross-resistant to cyclophosphamide after cyclophosphamide remission induction in the 13762 rat mammary tumor system.[8] The results reported here in previously treated advanced-disease patients further support the theory that Dbd is an active drug against breast carcinoma.

The side effects observed with the intermittent schedule appear to be less severe than those reported with the daily schedule used in the COG trial (6). Of particular interest was the decreased incidence of hepatic (one of 29 patients) and gastrointestinal (six of 29 patients) toxicity. The drug also appears to affect hemoglobin levels because transient drops occurred in 11 of 48 cycles of therapy. The day and degree of the nadir wbc and platelet toxicities and the number of recoveries by Day 29 suggest that a dose of 180 mg/m²/day for 10 days repeated every 28 days is a tolerable regimen in previously treated patients. It would be expected

[8]Bogden AR. Personal communication.

that a higher dose, eg, 210 mg/m^2, would be well tolerated in patients not previously exposed to chemotherapy.

The clinical results suggest that Dbd should be tested in combination regimens for breast carcinoma. Combination regimens developed with this drug could provide a basis for either a second- or first-line combination to complement other current regimens. The present trial provides a baseline for developing combination regimens utilizing an intermittent schedule of therapy. Theoretically, Dbd could be utilized as a basic drug in the development of a non-cross-resistant regimen for fixed cross-over, or rotating, therapy in a primary treatment program. These considerations, and the observation that Dbd and adriamycin are synergistic in a C3H mouse tumor system,[9] have led us to combine Dbd with adriamycin in another phase I–II trial, the results of which will be reported separately.

[9]Griswold DP. Personal communication.

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5-Fluorouracil (5-FU), Methyl-CCNU, and Vincristine in the Treatment of Advanced Colorectal Cancer: Phase II Study Utilizing Weekly 5-FU [1,2,3]

John S. Macdonald,* Daniel F. Kisner, Tarilyn Smythe, Paul V. Woolley, Lee Smith, Jr, and Philip S. Schein [4]

SUMMARY

We report a phase II trial of 5-fluorouracil (5-FU), methyl-CCNU, and vincristine (FMV) combination chemotherapy in advanced colorectal carcinoma. Ten of 25 patients (40%) achieved an objective tumor regression after treatment with FMV chemotherapy. The main toxicities of FMV were hematologic and gastrointestinal. 5-FU was administered in a weekly rather than a loading-dose schedule which allowed for sensitive adjustment of drug dosages according to hematologic toxicity; only 16% of the patients (four of 25) had a wbc count < 2000 cells/mm^3. FMV is significantly more active than 5-FU alone in the treatment of advanced colorectal cancer; however this regimen does not significantly improve survival in responding patients.

[Cancer Treat Rep 60:1597–1600, 1976]

Since its development 18 years ago, 5-fluorouracil (5-FU) has remained the standard chemotherapeutic agent for the treatment of advanced colorectal cancer. In well-designed studies (1,2) 5-FU has been demonstrated to produce an objective regression of measurable disease in approximately 20% of the patients although there has been little evidence (3) that treatment with this agent has improved the overall survival rate.

During the last 10 years combination chemotherapy has markedly improved the outlook for successful treatment of patients with leukemia, lymphoma, and breast cancer. The results (3) of combination programs for large bowel cancer have until recently been quite discouraging. However, during the past 2 years Moertel et al (4) and Falkson (5) have reported that the combination of 5-FU, methyl-CCNU, and vincristine (FMV) is capable of inducing objective responses in approximately 40% of the patients with advanced colorectal cancer.

We report a phase II study in advanced colorectal cancer which utilizes FMV in a dose schedule designed to minimize myelosuppression. In our regimen, 5-FU is given as a weekly iv dose rather than an iv loading dose and we are able to demonstrate response rates comparable to those of Moertel et al (4) and Falkson (5) with the potential for less toxicity.

MATERIALS AND METHODS

FMV chemotherapy was administered in 6-week cycles (table 1). 5-FU was administered at weekly iv doses of 500 mg/m^2, vincristine was administered every 6 weeks in doses of 1.0 mg/m^2 iv, and methyl-CCNU was administered every 6 weeks at 150 mg/m^2 orally. Doses of 5-FU and methyl-CCNU were modified according to hematologic toxicity as measured by the wbc and platelet counts (table 2). Cbc counts were performed before each weekly treatment. Since the nadir of the myelosuppression induced by methyl-CCNU was seen 3–5 weeks after

[1]Received Aug 2, 1976; revised Sept 28, 1976; accepted Oct 7, 1976.

[2]Supported in part by contract N01-CM-67110 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8.

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

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TABLE 1.—FMV therapy administered in 6-wk cycles

Drug	Dose (mg/m^2)	Week No.— 1	2	3	4	5	6
Methyl-CCNU	150, orally	×					×
5-FU	500, iv	×	×	×	×	×	×
Vincristine	1.0, iv	×					×

drug administration, the next dose of this drug was adjusted according to this nadir count.

Twenty-five consecutive patients with metastatic colorectal cancer and followable disease were placed on FMV therapy. Table 3 details the clinical data on these patients. The ages ranged from 28 to 77 years with a median age of 66 years. All patients had their performance status (PS) assessed according to the Eastern Cooperative Oncology Group (ECOG) scale. The majority of the patients were ambulatory and there was not a significant difference in PS between responders and nonresponders (table 3). PR was defined as a decrease of ≥ 50% in the products of the two largest perpendicular diameters of a clearly measurable area of malignant disease chosen before treatment as the primary indicator lesion. There could be no increase in size of other known areas of malignant disease or appearance of new areas of malignant disease. The response must have been observed at least 3 weeks after the start of therapy. If hepatomegaly was chosen as an indicator lesion, a decrease of ≥ 30% in the sum of the measurements below the xiphoid process and both costal margins at the midclavicular lines was the criterion for response.

RESULTS

Ten of 25 patients (40%) achieved a PR to FMV chemotherapy. The median duration of response was 7.5 months (range, 3–12.5 months) and three of the ten responders remain in remission. Seven of the ten responders (70%) are dead; their median survival time will be > 9.5 months but will not exceed 15 months. Sixty-six percent of the nonresponders are dead. The median survival time for this group will be > 8.0 months but will not exceed 11 months. The differences in survival time are not statistically significant ($P = 0.64$).

FMV chemotherapy was generally well tolerated. Nausea was frequently noted on the first day of chemotherapy and could be attributed to methyl-CCNU. This was moderate to severe in three of 25 patients but in no patient was gastrointestinal (GI) toxicity so severe as to limit the amount of chemotherapy that could be administered. No drug-associated diarrhea or mucositis was encountered.

Hematologic toxicity was common with FMV chemotherapy (table 3). The median nadir of the wbc count was 3700 cells/mm^3 (range, 0.8–11.0 cells × $10^3/mm^3$) and only four of 25 patients (16%) had wbc counts < 2000 cells/mm^3. The median nadir of the platelet count was 60,000 platelets/mm^3 (range, 15–463 platelets × $10^3/mm^3$). Twenty-three of 25 patients required dose modifications of the myelosuppressive agents 5-FU and methyl-CCNU. The two patients who did not have myelosuppression from FMV chemotherapy (patients 17 and 25) had evidence of disease progression during the first cycle of therapy and chemotherapy with FMV was discontinued. Twenty-three of the 25 patients required modification of 5-FU dosage at 4–5 weeks of therapy and all patients (22 of 25) who received more than two courses of FMV therapy required reduction of the methyl-CCNU dose in subsequent cycles. Six patients received four or more courses of FMV. None of these patients was able to receive > 50% of the projected dose of methyl-CCNU in subsequent courses and delay of all therapy from 1 to 3 weeks was required to allow recovery from myelosuppression.

Vincristine toxicity was mild and limited to distal extremity paresthesia and hyporeflexia except for two patients (Nos. 3 and 10) who developed muscular weakness and footdrop. In both instances the patients were > 65 years of age and had received continuous chemotherapy for ≥ 10 months.

TABLE 2.—FMV therapy: dose modification of 5-FU and methyl-CCNU in response to myelosuppression

% drug dose	Cell counts ($cells/mm^3$) Wbc	Platelets
100	> 4000	> 100,000
50	3000–3999	75,000–99,999
None	< 3000	< 75,000

TABLE 3.—Patient data

Patient No.	Age/sex	Site of metastatic disease	PS (ECOG)	Nadir wbc count (cells × 10^3/mm^3)	Nadir platelet count (platelets × 10^3/mm^3)	Response	Duration of response (mos)	Survival (mos)
1	55/M	Para-aortic nodes	2	2.6	78	PR	4.5	15.5
2	69/M	Liver	1	2.4	40	PR	5.0	15.0
3	69/F	Pelvis	1	1.5	15	PR	12.5	16
4	51/M	Pelvis, liver	2	2.2	176	PR	5.0	8.0
5	68/M	Liver	0	2.8	65	PR	11.5+	11.5+
6	49/M	Liver	1	4.3	98	PR	9.5+	9.5+
7	76/F	Liver	1	5.4	50	PR	9.5+	9.5+
8*	69/M	Abdominal tumor, pulmonary	3	1.6	34	PR	3.0	3.0
9	28/M	Abdominal tumor	2	0.8	57	PR	6.5	7
10	70/M	Liver, lung	1	3.7	56	PR	10	14.5
11	66/M	Liver	1	3.8	85	NR	—	8+
12	61/F	Liver, lung	1	3.9	24	NR	—	11
13	56/M	Pelvis, bladder	0	1.6	75	NR	—	11
14	77/M	Lung	0	4.7	35	NR	—	8.0
15	55/M	Pelvic nodes	3	5.8	26	NR	—	1.5
16	63/M	Abdominal tumor, liver	1	2.6	139	NR	—	7.0
17	39/M	Para-aortic nodes	0	11.0	220	NR	—	4.5
18	59/F	Liver	2	2.7	56	NR	—	4.0
19	69/F	Bone, liver	0	4.9	20	NR	—	8+
20	50/F	Liver	0	3.5	85	NR	—	11
21	70/F	Liver	0	3.7	60	NR	—	8.5+
22	77/M	Liver, pelvic tumor	1	4.6	60	NR	—	13+
23	65/F	Liver	2	4.0	73	NR	—	6.5
24	74/M	Liver, unresected primary	2	3.4	36	NR	—	6+
25	72/M	Abdominal tumor, liver	1	3.4	463	NR	—	0.5

*Patient died while in response.

Two patients required hospitalization for drug-related complications. Patient No. 3 was admitted with fever and a wbc count of 1500 cells/mm^3 and was treated for 10 days with broad-spectrum antibiotics until the leukopenia resolved. Bacterial cultures remained negative during this febrile episode. The second patient (No. 8) requiring hospitalization fell and struck his head when his platelet count was 50,000 platelets/mm^3. This resulted in a subdural hematoma complicated by disseminated intravascular coagulation which led to the single drug-related death in this series.

DISCUSSION

In 1975 Moertel et al (4) reported a prospectively randomized trial between 5-FU and the combination of FMV in patients with metastatic colorectal cancer. Eighty patients were entered in their study and objective responses were seen in 19.5% of the patients treated with 5-FU alone whereas 43.5% of the patients treated with the combination of FMV responded. 5-FU was used in an iv loading-dose regimen with the drug being given at 10 mg/kg/day × 5 days in the combination-chemotherapy arm of the protocol. Significant toxicity was encountered with 21% of the patients developing mucositis and 37% of the patients having wbc count nadirs < 2000 cells/mm^3. This degree of significant toxicity may have been partially related to the loading-dose schedule used for 5-FU. Further confirmation of the efficacy of combination chemotherapy was provided by Falkson (5) in a randomized study of 91 patients with advanced colon cancer. Thirty-seven percent of the patients treated with FMV (17 of 46) exhibited an objective tumor shrinkage. Only 22% of the patients treated with 5-FU alone (ten of 45) responded. The median duration of response was 5.0 months in the combination-chemotherapy group and 3.8 months in the 5-FU group. Falkson's study utilized a loading-dose 5-FU regimen similar to Moertel's study and leukopenia was noted in 22 of 46 patients (47%).

Our phase II study of FMV chemotherapy in advanced colorectal carcinoma has demonstrated an objective response rate of 40% which corresponds to that seen by Moertel et al (4) and Falkson (5). We feel that the use of weekly 5-FU rather than loading courses of 5-FU makes FMV a potentially safer

chemotherapy program since one may modify the weekly 5-FU dosage according to hematologic toxicity. This flexibility offers an important advantage since the nitrosourea antitumor agents are known to produce delayed and cumulative myelosuppression (6).

Single-agent data with 5-FU suggest that the iv loading-dose schedule is more effective than a weekly schedule. The Central Oncology Group (COG) (7) has reported that loading-dose 5-FU is able to produce objective regression in 38% of the patients with metastatic GI and breast malignancies. Only 12% of the patients treated with weekly iv 5-FU responded. However, it should be emphasized that the COG program examined the effect of 5-FU as a single agent and that the results may not be directly transferable to use of this drug in combination programs. Data supporting this assumption have recently been reported from the Southwest Oncology Group (8). In a randomized study of chemotherapy of advanced GI cancer, it was shown that 9.5% of the patients with colorectal cancer responded to weekly 5-FU whereas 31.8% of the patients responded to weekly 5-FU in combination with methyl-CCNU given every 6 weeks. While our FMV study represents an uncontrolled phase II program, it is clear that the observed response rate of 40% is equivalent to the response rates reported for similar regimens utilizing loading-course 5-FU (4,5). Our results indicate that weekly 5-FU may be as effective as loading-dose 5-FU in the FMV regimen.

In our study it is important to emphasize that a dose of 150 mg/m^2 of methyl-CCNU administered every 6 weeks in the FMV program was difficult to deliver consistently because of cumulative myelosuppression. In response to this problem, we have modified the projected doses of methyl-CCNU to be used in our subsequent protocols. We currently use a maximum dose of 130 mg/m^2 of methyl-CCNU every 10 weeks in combination-chemotherapy studies.

The median survival time of the 25 patients in this study is 8.0 months (range, 0.5+–16+ months). This figure is longer than that reported by other investigators (2,5) and may result from the relatively good PS of patients entered into the FMV study.

The overall symptomatic improvement and observed response rate of 40% for FMV combination chemotherapy represents an improvement on past experience with single-agent chemotherapy. However, our experience and that of others (4,5) indicate that it is unlikely that the FMV regimen will significantly improve survival in patients with advanced colorectal cancer. To develop more effective regimens in patients with advanced disease, it is necessary to establish the activity of new single agents in this disease through phase II trials. Active agents must then be utilized in the design of effective combination regimens. If regimens similar to FMV are to make an impact on survival in patients with colorectal carcinoma, it will require that these combinations be used in patients with minimal residual disease as surgical adjuvant chemotherapy in controlled prospectively randomized trials.

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Antitumor Activity of 1,2-Bis Diazoacetyl Ethane[1,2]

N. M. Emanuel,* N. P. Konovalova, and R. F. Djachkovskaya[3]

SUMMARY

1,2-Bis diazoacetyl ethane (diazane) was selected for a detailed study as the most active compound in a large group of mono- and bi-functional diazoketones subjected to screening. In this paper data on the toxicity and cumulative and antitumor effects of diazane are reported. Diazane markedly inhibits the growth of certain experimental tumors and increases the mean survival time of animals with leukemias, even after a single injection. The drug is characterized by a high level of accumulation in the body.

[Cancer Treat Rep 60:1601–1604, 1976]

TOXIC AND CUMULATIVE PROPERTIES

The toxicity of 1,2-bis diazoacetyl ethane (diazane) (fig 1) was studied in a single-dose experiment using randomly bred mice weighing 18–20 g, C57BL mice of the same weight, and randomly bred rats weighing 100–200 g. Each animal was given a single ip injection of the drug and five to six different doses were tested. Multiple-dose toxicity was studied in randomly bred mice and rats using different routes of administration.

It has been found that when injected daily diazane shows a high cumulative effect. With that in mind we determined the LD50 for daily injections and for a schedule with 72-hour intervals between injections.

Toxic doses and the cumulative index values (J_k) are presented in table 1. The cumulative index was calculated from the equation:

$$J_k = \frac{LD50(1)-LD50(n)}{LD50(n)-(n-1)} \times 100$$

It can be seen from table 1 that the toxicity of diazane is low when the drug is given as a single injection; at the same time toxicity markedly increases when the drug is administered daily for a period of 7 days. When diazane is given at 48- and 72-hour intervals the tolerance increases markedly. For example, the maximum tolerated dose (MTD) for mice given daily ip injections is 0.5 mg/kg; when the drug is given at 72-hour intervals the MTD is 75 mg/kg. This is accompanied by a corresponding decrease in the cumulative index. Keeping in mind the marked cumulative effect of diazane in chemotherapeutic experiments, the drug was administered at 72- and 48-hour intervals.

ANTITUMOR EFFECT OF DIAZANE

Materials and Methods

The following mouse tumor strains were used: L1210 leukemia, La leukemia, Harding-Passey melanoma, Hepatoma 22, and Adenocarcinoma 755. Rat tumors studied were Sarcoma 45, Walker 256 carcinosarcoma, and Guérin carcinoma.

A total of 10^6 or 10^8 leukemic cells were inoculated when transplanting L1210 and La leukemias respectively; solid tumors were transplanted with ground cell suspensions in a volume of 0.3–0.5 ml. Diazane was given to animals (ten animals per group) on the day after tumor transplantation or later when the tumor had already developed. The drug was administered ip for solid tumors and sc

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TABLE 1.—Toxicity of diazane on ip injection*

Schedule of administration	LD50 (mg/kg)	Cumulative index
Mice		
One injection	760	
Daily, 7 injections	1.3	9730
7 injections given at 72-hr intervals	90	124
Rats		
One injection	150	
Daily, 7 injections	3	816
7 injections given at 72-hr intervals	12	190

*4 animals were used for each dosage level with a dose range of 100–1000 mg/kg in increments of 50 mg.

for ascites tumors. In La leukemia the routes of administration used were ip, sc, or oral; three to four injections were given to mice and five to six injections were given to rats. In testing diazane in Guérin carcinoma and Sarcoma 45 a dose of 4 mg/kg was given at 48-hour intervals. Diazane was initially given at a massive dose of 20 mg/kg; thereafter, maintenance doses of 5 mg/kg at 48-hour intervals were given. For L1210 and La leukemias diazane doses of 20–50 mg/kg were given and for solid tumors doses of 15–40 mg/kg were given. Moreover, the action of a single massive dose and repeated massive doses administered at 72- and 120-hour intervals in La leukemia was studied, with two to five experiments conducted for each dose of diazane.

The percent inhibition of tumor growth was used as the criterion of activity; it was determined from the weight of subcutaneous tumors in animals treated with the drug in comparison with the untreated animals. To determine the antileukemic activity, a kinetic method of evaluation by means of a calculated coefficient of inhibition (γ) was used. The coefficient γ shows the extent of tumor process inhibition compared with control (1). The mean survival time for treated animals compared with control animals was also used for evaluation of the antileukemic effect.

Results and Discussion

Diazane appears to have a high antileukemic activity. In this connection a detailed study of its effect on La leukemia was undertaken. La leukemia represents an acute generalized disease with stable characteristics, resistant to various forms of treatment. The kinetic characteristics of diazane were determined making use of this model.

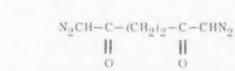

FIGURE 1.—Structure of diazane.

Figure 2 represents kinetic curves of change in the spleen weight of control animals and in animals treated with high doses of diazane (a change in the spleen weight indicates the development of the leukemic process). Diazane at a dose of 150 mg/kg was administered. In order to establish the relationship between antitumor activity and number of injections, experiments administering one, two, three, or four injections were carried out.

It is clear from figure 2 that the coefficient of inhibition increases with an increase in the number of injections; a single injection of diazane inhibits the growth of leukemia by a factor of 1.8. Further experiments showed that diazane administration at doses of 20–25 mg/kg with 72-hour intervals is optimal. Figure 3 represents kinetic curves of leukemia development obtained from administering diazane ip, sc, or orally. The inhibitory effects of diazane were mostly evident in cases of ip administration ($\gamma = 5.9$), although other modes of administration were also effective. For sc and oral administration the inhibition coefficients were 4.5 and 3.5 respectively.

The leukocyte count in treated animals remained normal and leukemic cells were not found either in the blood or in the bone marrow by 17–18 days. The number of leukocytes increased and single leukemic cells appeared only later.

La leukemia is known to be characterized by a marked resistance to chemotherapeutic treat-

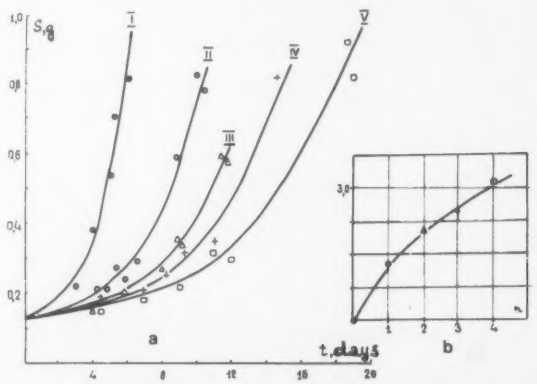

FIGURE 2.—(*a*) Kinetic curves for changes in the spleen weight in control animals (I) and in animals treated with diazane (150 mg/kg) with 1, 2, 3, and 4 injections (curves II–V respectively); (*b*) relationship between the inhibition coefficient and the No. of diazane injections.

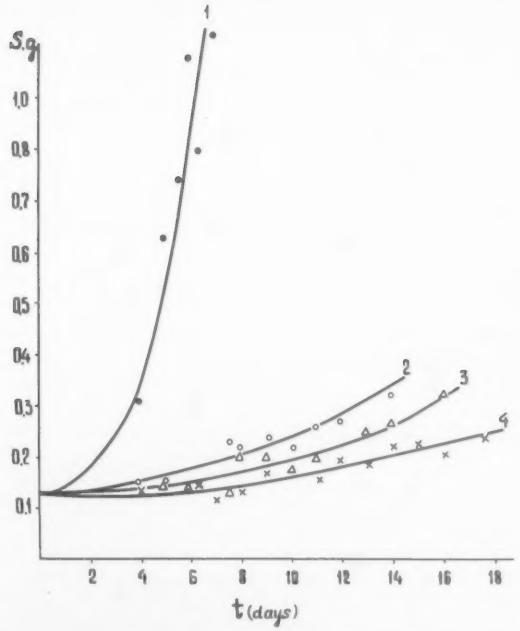

FIGURE 3.—Kinetic curves for La leukemia development due to the action of diazane administered ip (4), sc (3), and orally (2); control curve (1).

ment. A considerable antileukemic effect similar to that observed for diazane was obtained only in rare cases (2). In particular, the effect of diazane was manifested by an inhibition of spleen weight and an increase in the leukocyte count during the first 15 days of treatment. These parameters increased with time. It was of interest to elucidate the character of the changes in spleen weight and leukocyte count under the action of diazane in the course of leukemia development. The kinetic method used in these experiments permitted a clearcut picture to be obtained of the immediate effect of diazane administration.

Diazane was injected at a dose of 150 mg/kg/injection at 5-day intervals. The first injection was given 3 hours after transplantation of leukemic cells.

Figure 4 shows that there was a decrease in both the spleen weight and in the leukocyte count in the course of treatment. Kinetic curves for changes in the spleen weight and leukocyte count consist of sections for exponential growth and fall, and all changes connected with alternation of treatment and rest are clearly seen. After the first administration of diazane the increase in the spleen weight and leukocyte count was slower than in controls. After each subsequent administration (on Days 5 and 10 after

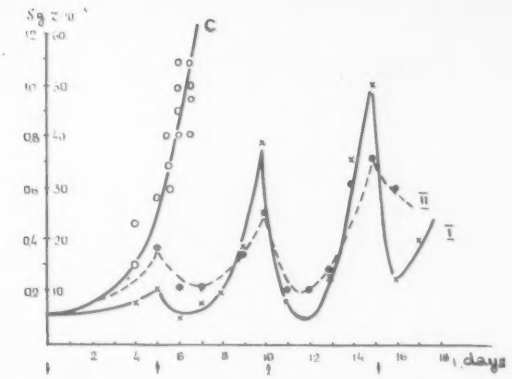

FIGURE 4.—Changes in indices of the leukemic process under the action of diazane (150 mg/kg). Interval between administrations is 5 days. I = changes in the spleen weight due to the action of diazane; II = changes in the leukocyte count due to the action of diazane; C = changes in the spleen weight in controls; ↑ = days diazane was injected.

transplantation) a marked decrease in both the spleen weight and the leukocyte count was observed.

After the last administration the spleen weight also considerably decreased; however, there was no response of leukocyte counts. Figure 4 shows that each decrease of the parameters studied to approximately normal values lasted for about 2 days, and was followed by an increase in the spleen weight and the leukocyte count.

Numerical values of constants calculated by the method of linear regression and characterizing the rate of the process at the sections of rise and fall change with time (table 2).

It can be suggested that with an increase in the total dose of the drug, the immunodepressive properties of diazane begin to be evident; this has an effect on the increase in the numerical values of constants at the sections of rise of the kinetic curves. It can possibly be accounted for by certain habituation to the drug. However, the rate of the

TABLE 2.—Values of constants (γ) for sections of growth and regression under the action of diazane

Sections of growth	Coefficient of inhibition (γ)
1	0.35
2	0.86
3	1.47
1	−0.98
2	−1.73
3	−2.06

TABLE 3.—Average survival time of animals with L1210 and La leukemias

Tumor	Drug	Total dose (mg/kg)	Hrs after transplantation that treatment was started	Average survival time (days)
La leukemia*	Control	—	—	6.5 ± 0.14
	Diazane	220	3	22.4 ± 2.0
	Diazane	140	24	14.3 ± 1.18
	Diazane	100	48	14.0 ± 2.4
L1210 leukemia†	Control	—	—	6.5 ± 0.2
	Diazane	140	24	13.2 ± 0.93

*10^6 leukemic cells inoculated ip.
†10^6 L1210 ascites cells inoculated ip.

TABLE 4.—Effectiveness of diazane (administered ip) in animals with early or advanced transplanted tumors*

Tumor	Treatment schedule (days)	Total dose (mg/kg)	Tumor weight, treated/control (g)	$\frac{T-C}{C} \times 100$†	No. of survivors/total treated
Sarcoma 45	1, 3, 5, 7, 9	25	1.3/13.2	91	3/10
	7, 9, 11, 13	35	1.8/13.2	86	1/10
Guérin carcinoma	5, 7, 9, 11, 13	25	4.4/15.2	71	0/10
	9, 11, 13, 15	35	4.2/15.2	72	0/10
Walker 256 carcinosarcoma	1, 4, 7, 9	35	0.9/16.9	95	3/10
	5, 7, 9, 11	35	1.8/16.9	89	1/10
Hepatoma 22	1, 3, 5, 7, 9	75	0.3/2.7	90	2/10
	5, 7, 9, 11	80	0.7/2.7	74	0/10
Adenocarcinoma 755	1, 4, 7, 10, 13	100	0.5/1.7	70	0/10
	7, 10, 13	75	1.2/1.7	29	0/10
Harding-Passey melanoma	—	—	—	—	—
	14, 16, 18	120	1.6/3.3	51	0/10

*Tumors were transplanted sc with cell suspensions in a volume of 0.3–0.5 ml.
†$\frac{T-C}{C} \times 100$ = retardation of increase in tumor weight in %.

process at the sections of fall was increased as well. The high cumulative ability of the drug obviously intensifies the antitumor effect. This effect may also be connected with modifications in proliferation kinetics of leukemic cells; in other words, the real rate of the process development can be a consequence of various combinations of many factors determining final therapeutic effect. Such a kinetic approach permits observing the course of the process determining the optimal time for changing the schedule and the dose, or substituting one drug for another with a different mechanism of action.

The antileukemic effect of diazane was also confirmed by its action on the increase in survival time of animals with La and L1210 leukemias (table 3).

Evaluation of diazane activity using other models demonstrated a wide antitumor spectrum of the drug. As shown in table 4 high indices of antitumor activity, with both early and late treatment, were obtained. In most cases the percent inhibition was 70%–100%. The experimental results obtained permitted recommending diazane for clinical trial. Presently, the first stage of clinical study is completed.

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Toxicity, Antitumor Activity, and Pharmacokinetics of Spin-Labeled ThioTEPA Analogs[1]

N. M. Emanuel,* N. P. Konovalova, and R. F. Djachkovskaya[2]

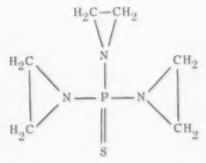
SUMMARY

Two spin-labeled analogs of thioTEPA, containing an iminoxyl radical, have been shown to have significant antitumor activity in several experimental tumor systems, combined with a marked decrease in toxicity compared with the parent compound, thioTEPA. Walker 256 carcinosarcoma, Guérin carcinoma, and Schweitz erythromyelosis showed complete regression using one of these analogs. Pharmacokinetic studies have shown that these compounds possess particular affinity towards tumor tissue.

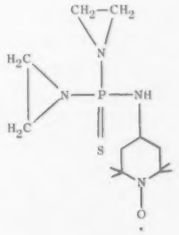
[Cancer Treat Rep 60:1605–1609, 1976]

Structural modification of various chemotherapeutic agents often results in marked changes in their biologic activity and therapeutic properties. Seemingly insignificant modifications in the chemical structure frequently decrease the toxicity of a drug, simultaneously increasing its effectiveness and selectivity. Moreover, insertion of particular chemical groups into a drug molecule may provide insight as to its body distribution and its mechanism of action at the molecular level. Recently, interest has focused on certain stable free radicals that are used as spin labels to study the interactions of chemicals with enzymes, nucleic acids, or proteins and for detecting the drug or its metabolites in blood, plasma, urine, or body organs (1–3).

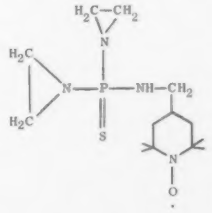
Since antitumor activity has been reported in some stable free radicals (4), we were interested in the antitumor activity of two paramagnetic analogs of thioTEPA (PAT-I and PAT-II). These were synthesized by the substitution of an iminoxyl radical for one of the ethyleneimine groups in thioTEPA (5). These compounds are the first reported spin-labeled antitumor agents.



ThioTEPA



PAT-I



PAT-II

The present report compares the toxicity and antitumor activity of these agents and the kinetics of the tissue distribution of PAT-I in normal and tumor-bearing animals.

MATERIALS AND METHODS

Randomly bred mice were used for the single- and multiple-dose toxicity studies. The following tumor

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strains were used for the antitumor activity studies: Adenocarcinoma 755, Walker 256 carcinosarcoma, Guérin carcinoma, and Sarcoma 45 implanted sc; ascites forms of Sarcoma 180 and Ehrlich carcinoma; and La leukemia and Schweitz erythromyelosis implanted ip. ThioTEPA was dissolved in water and its analogs in a solution of 10% ethanol in water. The drug was administered sc in the ascites-bearing animals while ip injections were used for the leukemias and solid tumors. The effectiveness was evaluated by the percent inhibition of tumor growth and by a kinetic criterion, inhibition coefficient (γ) (6).

Electron paramagnetic resonance (ESR) was used to study the pharmacokinetics of PAT-I in the blood, liver, and tumors of mice bearing Adenocarcinoma 755 and the blood and liver of normal controls. A total of 150 mg/kg of body weight of PAT-I was injected ip 10 days after tumor transplantation and organs were removed at timed intervals. Blood was removed from the tail vein. Livers and tumors were removed and homogenized.

Since the iminoxyl radical undergoes reversible reduction in vivo, an oxidizing mixture, which increases the measurable amount of material by a factor of > 25, was added to the tissue samples. The oxidant, containing 30% H_2O_2, 1 N KOH, and water in the ratio of 2:1:1, was added to an equal volume of tissue homogenate and the mixture transferred to capillaries with a diameter of 1.1 ± 0.05 mm.

An improved spectrometer, a modification of ESR-2 (7) with an HF modulation frequency of 117 kc and a sensitivity of 10^{11} ersted, was used (signal ratio 10:1) at room temperature. Standards consisted of 1 volume of PAT-I solution to 1 volume of the oxidizing mixture. The concentration of free radicals in biologic samples was calculated from the normalized signal amplitude to the amplification ratio. The ESR spectrum of the thioTEPA spin-labeled analog represents a triplet with splitting between the components at 15–16 ersted (fig 1). The ratios of the amplitudes of these components were similar in all the tissues studied.

RESULTS AND DISCUSSION

Table 1 summarizes the comparative toxicity of thioTEPA, PAT-I, and PAT-II. Both analogs show a marked reduction in toxicity in both injection schedules, especially PAT-I in which it is decreased by an order of magnitude compared with thioTEPA. It is noteworthy that the slight alteration in structure (introduction of a CH_2- group) in PAT-II reverses the magnitude of this effect.

The antitumor activity of PAT-I is greater than that of either thioTEPA or PAT-II (table 2). Guérin carcinoma, Walker 256 carcinosarcoma, and Schweitz erythromyelosis undergo complete regression using PAT-I therapy. Kinetic estimations of the antitumor effectiveness of the agents studied have shown that PAT-I has a higher activity compared with the other two compounds. Figure 2 shows the kinetic curves for La leukemia development in animals treated with thioTEPA and its analogs and in untreated controls. The inhibition coefficients showing the extent of inhibition of the leukemic process by PAT-I, thioTEPA, and PAT-II are 1.9, 1.4, and 1.2 respectively. A comparison of these values indicates that the highest antileukemic activity was achieved using PAT-I.

Figure 3 shows the kinetics of the changes in the mean diameter of Adenocarcinoma 755 in untreated tumor-bearing animals and in the course of treatment starting 5 days after transplantation, when the tumor was already advanced and had a diameter of 0.4 cm. PAT-I was most effective while PAT-II was the least effective.

Since PAT-I has the higher antitumor activity, its effect on the kinetics of Schweitz erythromyelosis regression was studied in detail. ThioTEPA and PAT-I, administered in doses equimolar with respect to the amount of ethyleneimine groups, brought about tumor regression on Day 8 following tumor transplantation. Figure 4a shows that tumor regression begins immediately upon administration of PAT-I while in the case of thioTEPA the tumor continues to grow for at least 1 day. The rate of

TABLE 1.—Toxicity of thioTEPA and its spin-labeled analogs

Compound	LD50 (mg/kg)		MTD* (mg/kg)	
	Single dose	Multiple dose†	Single dose	Multiple dose†
ThioTEPA	18	6	12	2
PAT-I	187	32	150	10
PAT-II	40	15	20	10

*MTD = maximum tolerated dose.
†Mice were injected once daily for 10 consecutive days.

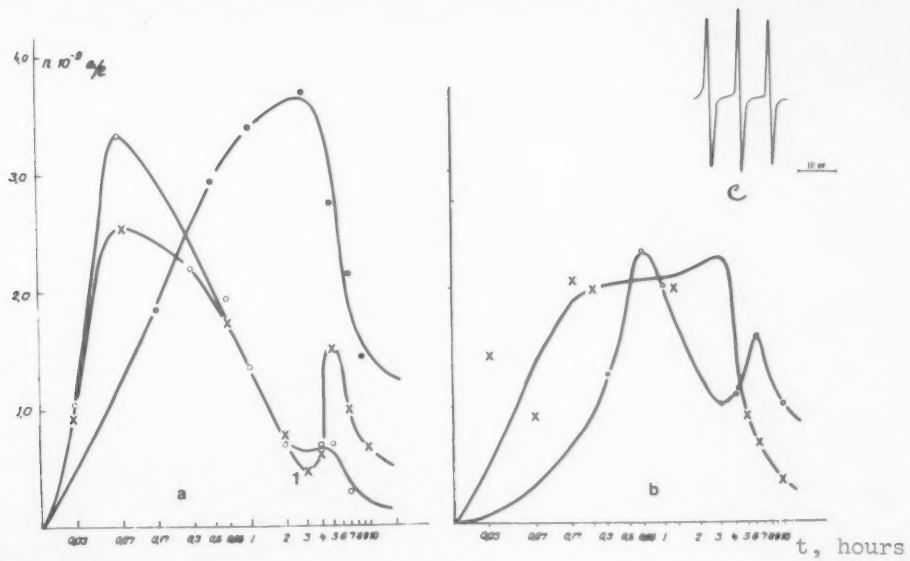

FIGURE 1.—Kinetics of the changes in label concentration in tumor (●), blood (×), and liver (○) of animals with Adenocarcinoma 755 (*a*) and in normal controls (*b*) after administration of PAT-I as a single dose of 150 mg/kg ip. (*c*) ESR spectrum of PAT-I.

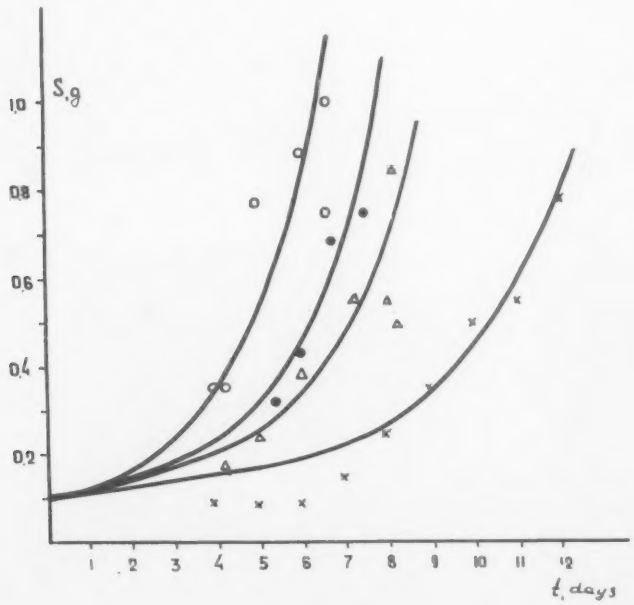

FIGURE 2.—Kinetics of La leukemia development. ○ = Control. ● = PAT-II at 10 mg/kg from Day 1 to 7. △ = ThioTEPA at 2 mg/kg from Day 1 to 7. × = PAT-I at 8 mg/kg from Day 1 to 7.

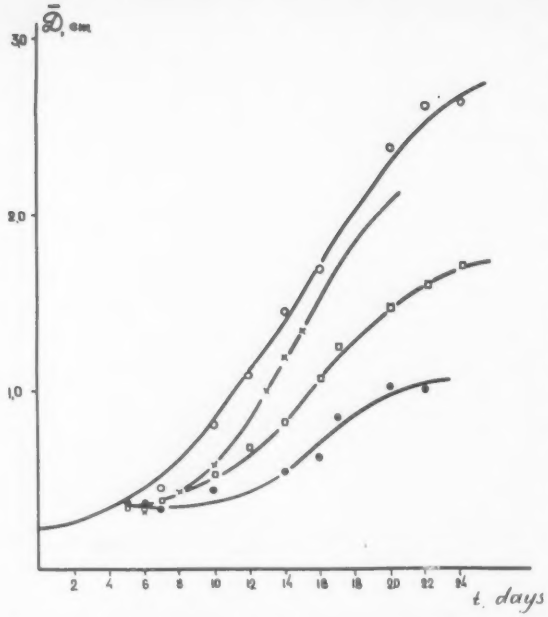

FIGURE 3.—Kinetics of the changes in mean diameter of Adenocarcinoma 755. ○ = Control. × = PAT-II at 10 mg/kg from Day 5 to 15. □ = ThioTEPA at 2 mg/kg from Day 5 to 15. ● = PAT-I at 20 mg/kg on Days 5, 7, 9, and 11.

regression was also somewhat different: the constants of steady decay were 0.11 and 0.15 days^{-1} respectively. A comparison of the regression constants shows that their differences are significant. The qualitative advantages of PAT-I over thioTEPA are undoubted. Complete regression of the tumor in animals treated with thioTEPA occurred much later than in those treated with PAT-I.

The rate of tumor regression using therapeutic doses of the agents are shown in figure 4b: PAT-I is more effective than thioTEPA, the regression constants being 0.35 and 0.1 days^{-1} respectively.

On the basis of the above data, the paramagnetic analog of thioTEPA can be regarded as a combination-action drug. Its antitumor activity can be accounted for by the ethyleneimine groups plus the iminoxyl radical fragment.

The kinetics of distribution of PAT-I in organs and tissues was studied for Adenocarcinoma 755 in animals. Figure 1 shows the kinetics of changes in the label concentration. A significant amount was found in all organs and tissues of the intact and tumor-bearing animals within 20 minutes after injection.

The concentration of PAT-I in tumor tissue increased over the first 3 hours following administration and then began to decrease. PAT-I showed a marked affinity towards tumor tissue compared with liver and blood. This is particularly interesting because thioTEPA itself does not possess this affinity, as has been shown using radioactive thioTEPA (8,9).

It must be emphasized that the maximum level of PAT-I in the tumor coincided with the minimum concentration in the blood and liver of the same animals. The differences between the drug level in the blood of control and tumor-bearing animals were quite noteworthy. In control animals the blood level of the drug remained constant over 3 hours whereas in tumor-bearing animals the blood level decreased rapidly, the minimal concentration of PAT-I being attained 3 hours after administration.

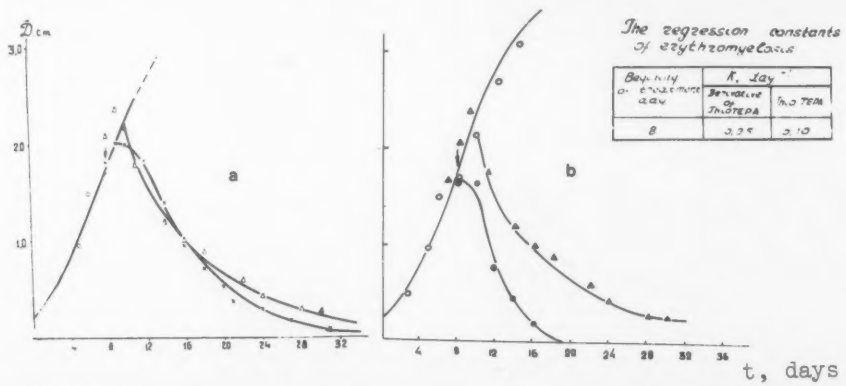

FIGURE 4.—Kinetics of the changes in mean diameter of Schweitz sc erythromyelosis after administration of thioTEPA and PAT-I in equimolar doses (*a*) and in therapeutic doses (*b*). (*a*) ● = Control. △ = ThioTEPA at 2 mg/kg from Day 8 to 18. × = PAT-I at 5 mg/kg from Day 8 to 18. (*b*) ○ = Control. △ = ThioTEPA at 2 mg/kg from Day 8 to 18. ● = PAT-I at 16 mg/kg from Day 8 to 12.

TABLE 2.—Antitumor effect of thioTEPA, PAT-I, and PAT-II on experimental tumors in mice and rats*

Compounds	Ascites tumors†		Solid tumors‡			
	Ehrlich tumor	Sarcoma 180	Adenocarcinoma 755	Walker 256 carcinosarcoma	Guérin carcinoma	Sarcoma 45
ThioTEPA	44	64	75	98	79	80
PAT-I	67	47	94	100	100	99.6
PAT-II	65	57	40	93	54	56

*Expressed as % inhibition or treated/control × 100.

†Ehrlich ascites tumor and Sarcoma 180 were treated with thioTEPA at 2 mg/kg from Day 1 to 8 after tumor implantation; PAT-I was given at 8 mg/kg and PAT-II at 10 mg/kg from Day 1 to 6 after tumor implantation.

‡Walker 256 carcinosarcoma, Guérin carcinoma, and Sarcoma 45 were treated with thioTEPA at 2 mg/kg from Day 5 to 15, PAT-I at 16 mg/kg on Days 5, 7, 9, and 11, and PAT-II at 6 mg/kg from Day 5 to 15 after tumor implantation.

In addition, the level of PAT-I in the blood of tumor-bearing animals showed a second maximum 5 hours after administration. At approximately the same time the maximum amount of radical content was found in the livers of both normal and tumor-bearing animals.

These kinetics might be accounted for by the slow formation of metabolite(s) of PAT-I with the structure of the spin label unchanged, and consequently the ESR spectrum remaining unaltered. The metabolite(s) gradually passes into the blood and may thus be responsible for the appearance of the second peak on the curve for the "label" content in the blood.

The results obtained in these studies demonstrate that PAT-I, which is characterized by low toxicity, high antitumor activity, and affinity towards tumor tissue, may be of interest from theoretical and practical points of view both for further clinical studies and for solving particular pharmacokinetic problems.

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Rapid Analytic Method for Adriamycin and Metabolites in Human Plasma by a Thin-Film Fluorescence Scanner [1,2,3]

Eric Watson and Kenneth K. Chan [4,*]

SUMMARY

A rapid and highly sensitive method is described for the quantitative determination of adriamycin and metabolites in plasma. Adriamycin (Ad) with daunorubicin added as the internal standard was extracted from plasma with chloroform-isopropanol (1:1), separated by thin-layer chromatography (TLC), and quantitated in situ via a fluorescence scanning technique. This method represents one of the very few reliable quantitative TLC methods and is suitable for the routine determination of Ad in plasma in amounts as low as 2 ng/ml. Plasma Ad levels at 24 hours in rabbits and in patients both given a 1-mg/kg dose, previously regarded as difficult to be measured, are now easily measurable using as little as 0.5 ml of plasma. Multiple samples (ten to 15) can be analyzed by this method within a day.

[Cancer Treat Rep 60:1611–1618, 1976]

Disposition and pharmacokinetic studies of adriamycin (Ad), a new anticancer drug isolated from *Streptomyces peucetius* var. *caesius*, necessitates the development of a sensitive and specific analytic method. Several analytic methods (1–8) have been developed for the determination of Ad in biologic materials. Inherent disadvantages, particularly the lack of specificity, exist in the total radioactivity as well as in the total fluorescence methods (1–3). Several of the previously developed chromatography-fluorescence methods (4–6) have employed hydrolysis of Ad to form the aglycone, adriamycinone, a procedure which obscured the determination of free adriamycinone and potentially heat- and acid-sensitive metabolites. Others, however, have extracted Ad and metabolites without hydrolysis from biologic sources for identification (7–12). In addition, manual scraping of a thin-layer spot from the plate was tedious and often caused considerable variability in the determination of Ad. This problem, however, has been slightly diminished through the use of a flexible silicic acid-impregnated glasswool sheet (5). The applicability of the radioimmunoassay method (13) is limited because cross reactivity with metabolites and variability exists. With those considerations in mind, a recently published procedure included separation of components by high-pressure liquid chromatography prior to the radioimmunoassay determination (14). However, only urinary Ad and its metabolites were determined by this procedure.

Recently developed fluorescence scanning is a highly sensitive analytic technique that has the advantage of quantifying a compound in situ after separation of the compound from interfering substances via thin-layer chromatography (TLC) to an R_f value characteristic of the compound and the solvent system. In addition, specific excitation and emission wavelengths of the fluorescent component further contribute to the specificity of the measurement. Since Ad had already been shown to be amenable to separation by TLC on silica gel, the technique of in situ fluorometry appeared to be ideally suited to its analysis in plasma.

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[2]Supported by contract N01-CM-23241 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8. Supplied by the Developmental Therapeutics Program (DTP), Division of Cancer Treatment (DCT), NCI.

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MATERIALS AND METHODS

All solvents were reagent grade (Mallinckrodt Chemicals, St. Louis, Mo). Glassware was silanized with dimethyldichlorosilane (Pierce Chemical, Rockford, Ill) before use. Because of the instability of Ad and daunorubicin (Da)[5] in aqueous solution over an extended period of time, stock solutions of these compounds were prepared weekly in distilled water and were stored refrigerated in the dark.

Silica gel plates (20 × 20 cm, 0.25 mm thick, E. M. Merck, Elmsford, NY) were scored into 16, 1 cm-wide lanes. Fluorescence scanning was performed on a spectrophotofluorometer equipped with a thin-film scanner (Aminco-Bowman, Silver Spring, Md). The background noise was reduced considerably by incorporating a commercial filtering device (Spectrum Scientific, Newark, Del) to the output which was displayed on a strip chart recorder (Perkin-Elmer, Norwalk, Conn).

Procedure

Da was added to 0.5-ml aliquots of human plasma as the internal standard. The sample was extracted once with 2-ml ice-cold chloroform-isopropanol (1:1). The lower organic layer was transferred to a 10-ml pyrex tube and was evaporated to dryness via a stream of nitrogen. The walls of the tube were washed with 3 × 10-μl portions of chloroform-methanol (1:1) and the solvent was gently evaporated. The residue was dissolved by adding about 3–4 μl of chloroform-ethanol (1:1) and the entire sample spotted on the silica gel plate using a 2-μl Lang Levy pipet. On each plate were spotted 11 unknown samples, one of which was a blank, and five spiked human plasma samples serving as a standard curve. The plate was first developed ascendingly to 12 cm from the origin in a lined tank containing solvent I (chloroform-methanol-acetic acid, 93:5:2). The plate was then removed, dried briefly (1 minute) using an air gun,[6] and placed in solvent II (chloroform-methanol-acetic acid, 76:20:4). Development height was again 12 cm from the origin. The plate was again dried briefly and allowed to stand in the hood for 10 minutes before scanning.

The excitation wavelength for the fluorescence scanning was set at 475 nm and emission at 580 nm. The spots in each lane were centered by manipulation of the *x-y* axis on the TLC scanner until the maximum fluorescence intensity was obtained on a selected internal standard spot. The plate was then returned to the origin of the spot and the scanning was carried out in the direction of the chromatographic development at a speed of approximately 2.2 cm/minute.

A standard curve of Ad:Da fluorescence peak height ratio versus concentrations of Ad was generated for each plate. The plasma levels of Ad (ng/ml) were obtained from this standard curve.

RESULTS AND DISCUSSION

The recovery of Ad and Da extraction from plasma was about 70%. This value was established

[5]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6. Supplied by the DTP, DCT, NCI.

[6]No appreciable difference was observed when drying with an air gun for 30 minutes was compared to drying with a stream of nitrogen.

TABLE 1.—R_f values of Ad and metabolites on the TLC* system

Compound (source)	R_f values for solvent†— I	I and II‡	I, II, and IV‡	IV only
Metabolite X (rabbit plasma)	0.078(0)§	0.125(0.005)§	0.311(0)§	0.294(0)§
Metabolite X (human plasma)	0.082(0)	0.125(0.005)	0.307(0.004)	0.296(0)
Adriamycinol (rabbit bile)	0.069(0)	0.121(0)	0.294(0.008)	0.299(0.004)
Ad (rabbit plasma)	0.078(0)	0.204(0.005)	0.468(0.001)	—
Ad (human plasma)	0.082(0)	0.209(0.001)	0.458(0)	—
Ad¶ (standard)	0.069(0)	0.204(0)	0.450(0)	—
Adriamycinone¶ (standard)	0.304(0.014)	0.778(0.027)	0.934(0.002)	—
Da¶ (standard)	0.069(0)	0.266(0.004)	—	—
Adriamycinol aglycone¶ (rabbit bile)	0.107(0.011)	0.506(0.017)	0.801(0.020)	—
Metabolite Y (human plasma)	0.181(0.009)	0.415(0.007)	0.900(0)	—
Metabolite Z (human plasma)	0.350(0.021)	0.657(0.013)	0.943(0)	—
Metabolite Y (rabbit plasma)	0.174(0.002)	0.420(0.002)	0.904(0.004)	—
Metabolite Z (rabbit plasma)	0.367(0.004)	0.670(0.004)	0.951(0)	—

*On Merck silica gel-60 precoated plate.

†Solvent I = chloroform-methanol-acetic acid, 95:5:2, vol:vol:vol; solvent II = chloroform-methanol-acetic acid, 76:20:4, vol:vol:vol; solvent IV = chloroform-methanol-acetic acid-water, 80:20:14:6, vol:vol:vol:vol.

‡After consecutive runs of the solvent systems in the order indicated.

§Average of minimum of 3 values; values in parentheses are standard deviations.

¶Spiked into human or rabbit plasma.

by comparing the fluorescence peak heights obtained by scanning the TLC plates containing the spiked plasma extract with those obtained by scanning the plates on which the same amounts of Ad and Da had been directly applied.

Appropriate TLC chromatograms for plasma interference, resolution, stability, specificity, and reproducibility evaluations of this method are shown in figures 1–5. The R_f values of Da, Ad, and Ad metabolites are shown in table 1. The excitation and emission spectra of Ad and Da measured on TLC plates are shown in figure 6. The standard curve plotted by the relative fluorescence ratios of Ad to Da versus concentrations of Ad is shown in figure 4. Ad and Ad metabolite plasma levels of three rabbits and two patients as determined by this method are shown in figures 5 and 7.

Choice of Internal Standard

One of the major problems in the in situ fluorometric measurement of the TLC method has been the quantitative spotting on the TLC plate. The introduction of a suitable internal standard can eliminate this particular problem. Da was selected as our internal standard for three reasons. First, since the chemical structure of Da is very similar to Ad, when added to plasma, both compounds can be extracted to about the same percentage by the proper selection of a solvent system. Second, Da was found to be easily resolved from Ad on the TLC system used and, in addition, plasma extracts contain no interfering substances near its R_f value (fig 1). Third, Da has the same structural features responsible for fluorescence (5,15), thereby eliminating the need for manipulation of the wavelengths and standardization of the instrument. In fact, the emission and excitation spectra of Da and Ad on the TLC plate matrix were found to be identical (fig 6).

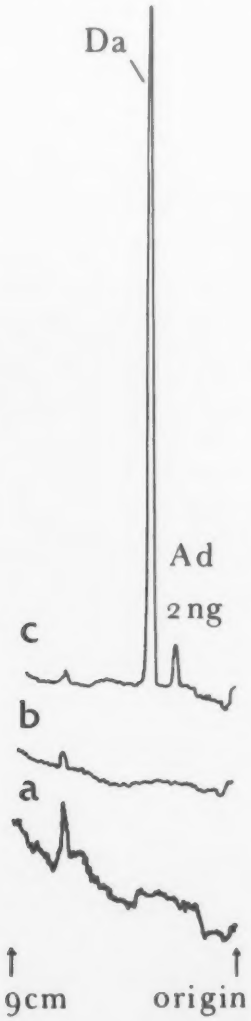

FIGURE 1.—TLC fluorescence scannograms: *a* = plasma blank, photomultiplier at 1, recorder at 50 mv, gain at 5, cut-off frequency at 0.1 MHz; *b* = same, with photomultiplier setting at 3; *c* = Ad- and Da-spiked plasma, same setting as *b*.

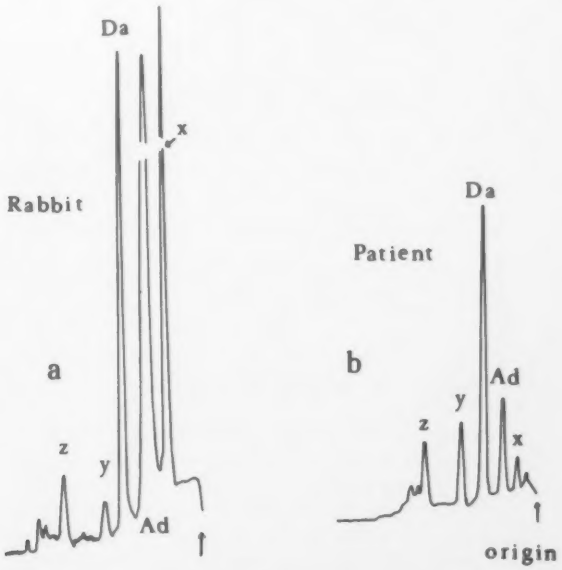

FIGURE 2.—TLC fluorescence scannograms of plasma extracts from a patient given 40 mg/m² and from a rabbit given 20 mg/kg: x = adriamycinol, y = unknown aglycone metabolite, z = unknown aglycone metabolite.

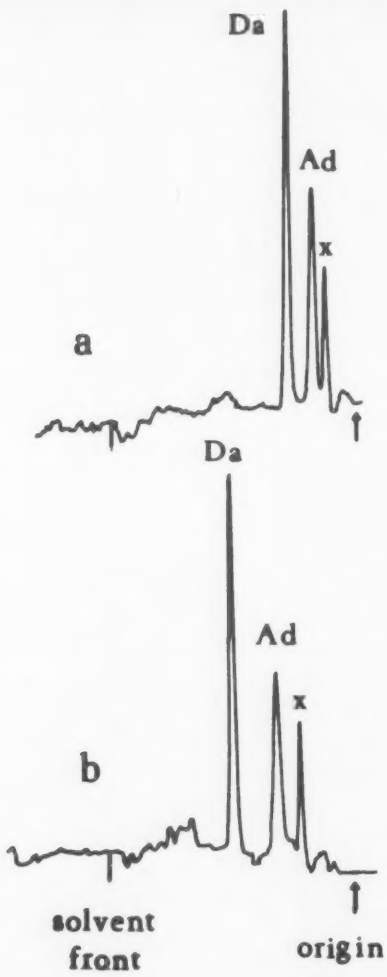

FIGURE 3.—TLC fluorescence scannograms of plasma extract developed through multiple TLC systems: *a* = after chloroform-methanol-acetic acid, 86:10:4, to 12 cm; *b* = same plate after chloroform-methanol-acetic acid-water, 80:20:14:6.

Interference and Sensitivity Evaluation

As shown in figure 1, no interference peaks with Da, Ad, and Ad metabolites were observed in the plasma blank and the signal to noise ratio at the low concentration (2 ng/ml) region was very high. The lower limit of detection of pure Ad is < 1 ng and occasionally similar results were obtained in spiked plasma. However, on the average runs of control human plasma and plasma specimens from patients, background values corresponding to 1 ng/ml of Ad equivalent have frequently been obtained. Therefore, the safe sensitivity limit is set at 2 ng/ml at present and is considerably more sensitive than several of the methods previously reported (3,5).

Resolution and Specificity Evaluation

In the initial development, the aglycones were first resolved by a solvent system (chloroform-methanol-acetic acid, 93:5:2; solvent I) and were scanned. Then a more polar combination of the same solvent system (86:10:4, solvent III) was subsequently used to resolve Ad and other more polar metabolites. It was later found that by the consecutive development of solvent systems I and II (*see* Procedure) all components were well resolved.

The resolution of Da, Ad, and Ad metabolites in the TLC system described was excellent as indicated by the TLC chromatogram (fig 2) and by the R_f values of these components (table 1). The resolution ranges from baseline separations to a < 10% valley between two adjacent peaks; the peak shapes were very symmetrical.

The specificity of a similar TLC fluorescence method for Ad has been previously evaluated (5). In order to evaluate this aspect of our method, various sources of human plasma extracts were scanned without the addition of Da and Ad, and no fluorescent component has been found to co-chromatograph with Da or Ad (fig 1). In an attempt to resolve any components that might be co-chromatographing with Ad, multiple developments have been carried out in the following solvent systems: solvents II, III, and IV (chloroform-methanol-acetic acid-water, 80:20:14:6). None of the chromatographic steps indicated that there were fluorescent components co-chromatographing with Ad and only resolution of the components was improved (fig 3). A change of basic media in the TLC system such as alumina or basic solvent resulted in decomposition of Ad and loss of fluorescence and is therefore of no value in the specificity evaluation.

No interference peak at the R_f values of Da was located when plasma from an Ad-treated rabbit was processed without addition of the internal standard.

The specificity of the measurement was also demonstrated by the characteristics of the excitation and emission wavelengths of those anthracyclines which had been previously discussed (5,15). These characteristics still remained in the in situ scanning (fig 6), and the emission wavelength was best chosen to be 580 nm, slightly different from that previously used (5).

Stability Evaluation

The stability of Ad and Da on the TLC plates

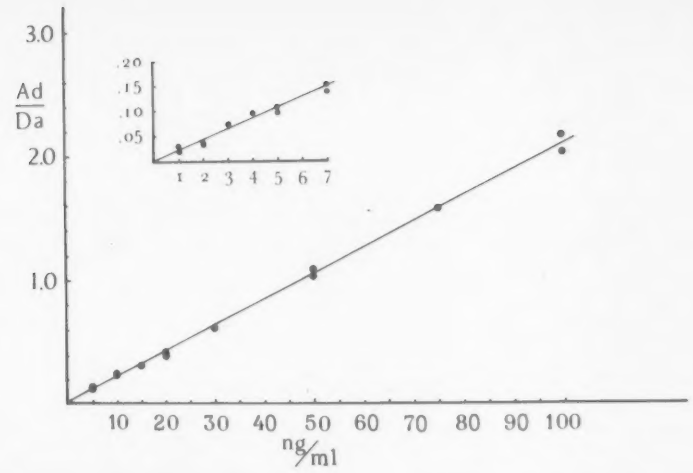

FIGURE 4.—Plasma standard curve of Ad. Horizontal axis represents Ad concentrations and vertical axis represents fluorescence peak height ratio between Ad and Da. Upper curve shows the linearity at low concentrations on a different plate. Due to the capacity limit, low- and high-concentration standard curves were performed on 2 separate plates.

after chromatography was of some concern in the initial stages of development of this method. A number of silica gel plates from commercial supplies were evaluated and it was found that the peak height of both Ad and Da rapidly decreased when the plates were left standing. The usual rate of decomposition was about 10% of the peak height every 10 minutes. Of the considerable number of commercial TLC plates evaluated, only two[7] were found to cause no decomposition of Ad and Da. Of these two, one[7,8] resulted in the best resolution of Ad and Da with minimum background.

Linearity and Reproducibility Evaluation

The standard curve from this method was usually linear in the range of 1–100 ng of Ad spotted (fig 4). However, infrequent deviation from linearity at about 75 ng was observed and could be attributed to the slight variability in the coatings on the TLC plates. For this reason, it was necessary to determine calibration curves for each plate by running standards along with each set of unknowns on the same plate. Still, this procedure allowed the analyses of up to ten unknown samples together with five standards on each plate, and analyses of 15 samples in duplicate can be done within the same day.

The reproducibility of the method was determined by the repeated analysis of a standard solution of 50 ng each of Ad and Da on the same plate. The fluorescence peak height ratio (Ad/Da) was found to be rather constant and the average value was found to be 0.907 ± 0.027 (eight analyses). The reproducibility of this method was also reflected by the excellent duplicates between determinations of the spiked plasma samples (fig 4) and of the plasma samples from two Ad-treated patients (fig 5). The average percentage deviation of these determinations was 3.9%.

Plasma Ad and Ad Metabolite Determinations

As shown in figure 2, Ad metabolites in small quantities were detected in rabbit and human plasma by this method. The lower ranges of metabolite concentrations expressed in Ad equivalents in these chromatograms were about 10 ng/ml. The slowest running spot, metabolite X, appeared to be the adriamycinol, presumably formed by enzymatic reduction of the carbonyl side chain of Ad.

The basis of the identification came from a direct comparison of the R_f value (0.50; Ad 0.73) of this component with that of the published R_f value (0.44; Ad 0.70) (10) using the same TLC system (silica gel H, chloroform-methanol-acetic acid-water, 80:20:14:6). The R_f values of this component (table 1) and those of a purified equivalent component obtained from the rabbit bile in several solvent sys-

[7]E. M. Merck and Quantum, Quantum Industries, Fairfield, NJ.

[8]No differences in results were found in > 5 different lots used.

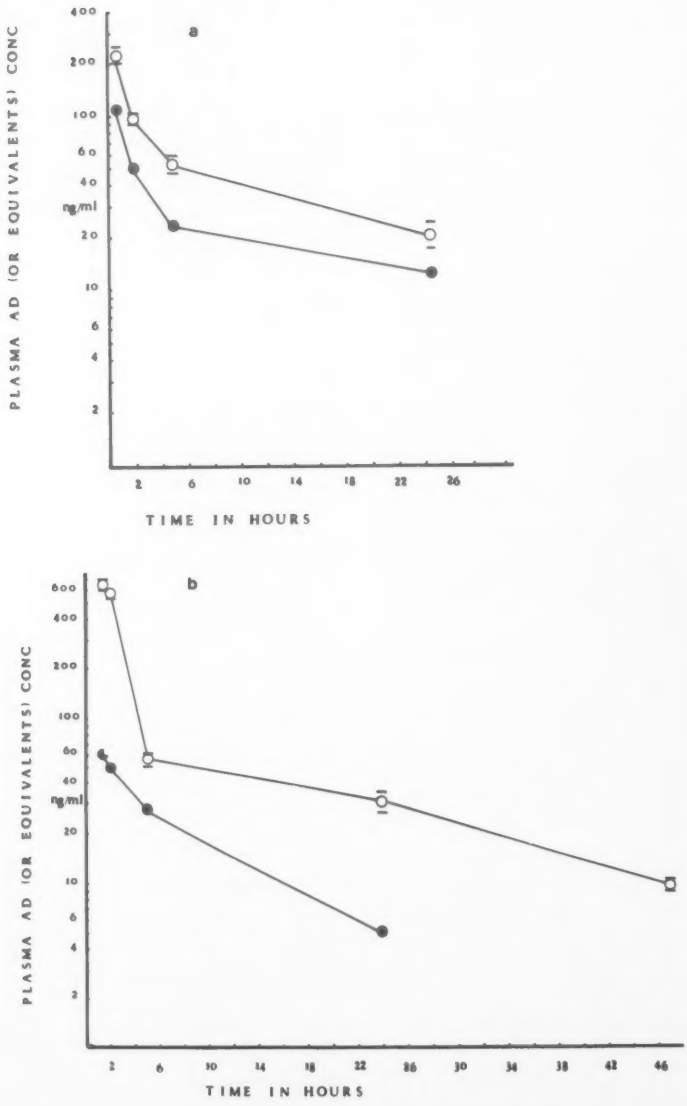

FIGURE 5.—Plasma Ad and adriamycinol levels in 2 patients given 60 mg/m² iv: ○—○ = Ad; ●—● = adriamycinol. The horizontal lines above and below the Ad points are the deviation from the mean of duplicates. *a* = patient No. 1, *b* = patient No. 2.

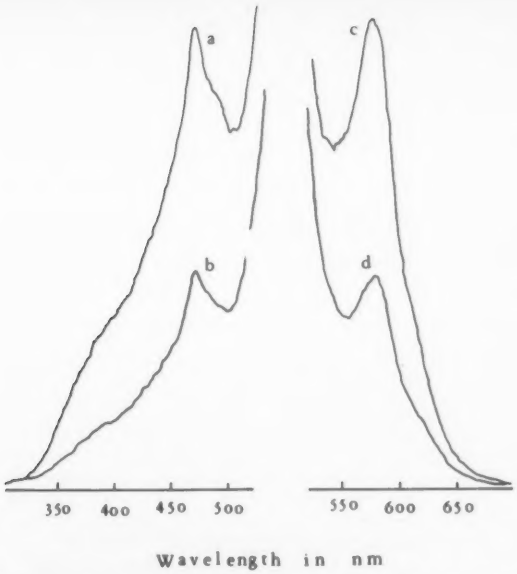

FIGURE 6.—Excitation and emission spectra of Ad and Da on TLC-plate matrix: *a* = Da excitation maximum at 474 nm; *b* = Ad excitation maximum at 474 nm; *c* = Da emission maximum at 580 nm; *d* = Ad emission maximum at 580 nm.

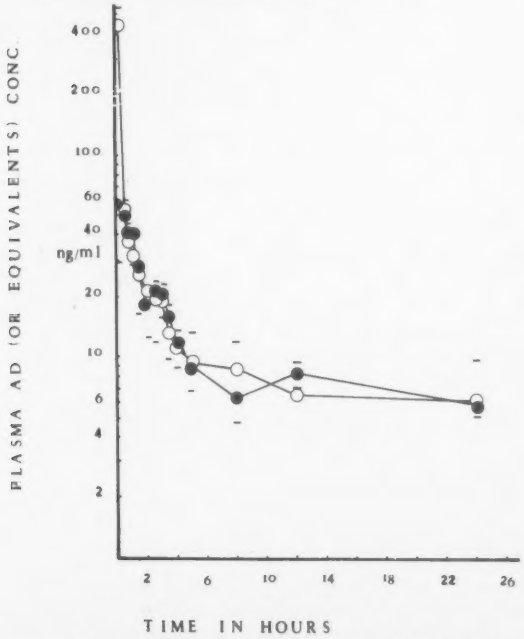

FIGURE 7.—Average plasma Ad and adriamycinol levels in 3 rabbits after 1 mg/kg iv: ○—○ = Ad; ●—● = adriamycinol. The top horizontal lines represent the standard deviations for the Ad level and the bottom lines represent the adriamycinol level.

tems (table 1) were practically identical. Since the amount of this metabolite that could be obtained from rabbit plasma is limited, more rigorous identification was performed on what appears to be the same component isolated from the rabbit bile. Mass spectral analysis of the aglycone of this metabolite, obtained from mild acid hydrolysis, exhibited a small but noticeable parent ion at m/e 416 and fragments at m/e 398, 380, and 349 identical to those reported (12).

A minor component[9] co-chromatographing with adriamycinol from the bile giving a parent at m/e 400 was also noted. The excitation and fluorescence scans of this spot in the TLC matrix gave respective wavelengths of 474 and 580 nm indicating essentially no change in chromophore.

Two of the fast running minor metabolites not present in the blank and having R_f values different from those of adriamycinone and adriamycinol aglycone (table 1) appeared only at high doses in rabbits (> 12 mg/kg) and were found to peak at about 25–40 ng/ml of adriamycin equivalent at a 20-mg/kg dose. Because of the limitation of the amount of these metabolites that could be obtained from rabbit plasma, no mass spectral analyses were performed on these components. In situ TLC fluorescence scanning indicated excitation and emission maxima identical with Ad. Because of the mobility in the TLC systems, these metabolites appear to be less polar than Da and are potential metabolite aglycones.

Similar metabolites were also detected in patient plasma (fig 2b) on the basis of the R_f values (table 1) compared with those of the rabbit. Metabolites y and z were detected only in certain patients. Since the quantity that could be obtained in human plasma is limited, only in situ excitation (474 nm) and emission (580 nm) spectra of these metabolites were obtained, which were essentially identical with Ad indicating no change in chromophore.

Ad and Ad Metabolite Plasma Levels in Rabbits and in Patients After Iv Injection

After iv administration of 1 mg/kg in rabbits, plasma Ad levels which previously were considered to be quite low for accurate determinations, especially at later times, are now easily measurable using the described method. The average parent drug levels in three rabbits (fig 7) decline rapidly from 437 ± 113 (± SD) ng/ml at 5 minutes to 6.0 ± 3.6 ng/ml at 24 hours.

[9] < 15% as verified by gas chromatographic-mass spectrometry. This component has not been identified as yet.

The semi-log plot of the concentration-time (C × t) curve appears to be multiphasic which is different than a biphasic profile reported in patients (6). The estimated half-life of one of the faster declines (5–60-minute intervals) was about 20 minutes and for one of the slower declines (5–24-hour intervals) was about 28 hours. The Ad level at 24 hours was still clearly well above the detectable limit of this assay method. The average metabolite, adriamycinol, levels varied from 54.7 ± 24.5 ng/ml at 5 minutes to 5.8 ± 0.72 ng/ml at 24 hours, with the similar rates of decline as the parent drug. Interestingly, with the exception of the earlier time points, plasma adriamycinol levels were comparable with those of the parent drug at this dose.

In two patients, both given doses of 60 mg/m², initial plasma concentrations (about 30 minutes) varied greatly (fig 5). A curvilinear plasma C × t curve appeared at the semi-log plot and the half-lives on one of the slower phases were in the range of 30 hours at a 60-mg/m² dose. The plasma levels of a major metabolite, adriamycinol, expressed as Ad equivalents, were considerably lower compared with those of the parent compound at all time points measured. No other metabolites in the plasma of these patients were detected in appreciable quantities (> 5 ng/ml).

Comparison With the Literature Method

Plasma Ad levels in a rabbit determined by this method were compared with those determined by a literature method (5). Comparable results (table 2) established the validity of this method.

CONCLUSION

Quantitative TLC analysis previously regarded as a rather unreliable method has recently been revived (16,17) due to the improvement in techniques and in material. However, this method is still far from perfect and many problems such as sensitivity and reproducibility remain to be improved. Very few methods have been available for drug analysis in biologic fluids. This paper described the analysis of Ad and Ad metabolites in human plasma and has demonstrated that by proper selection of internal standard, TLC materials, and fluorescence scanning technique, a reliable, sensitive, and specific TLC method can be applied to quantitatively analyze drugs in biologic fluids in a manner comparable to the other chromatographic methods.

TABLE 2.—Plasma Ad concentrations in rabbits as determined by 2 methods

Ad concentrations* in average ng/ml (SD)	
Described method	Hydrolytic instant TLC method (4)
625 (0)	660 (7.3)
503 (7.3)	490 (22.0)
285 (0)	270 (13.1)
136 (1.5)	190 (8.9)
95 (0)	76 (1.5)
83 (0.5)	80 (5.6)

*In triplicate.

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Combination Chemotherapy In Vitro With *Cis*-dichlorodiammineplatinum(II) [1,2]

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SUMMARY

***Cis*-dichlorodiammineplatinum(II) (DDP) was paired with 11 other chemotherapeutic drugs in a search for possible synergistic pairs. Cell lethality was investigated by the colony-formation technique utilizing a long-term culture of human lymphoma cells. Synergistic effects were noted for pair combinations with all alkylating agents, bleomycin, adriamycin, camptothecin, and cytosine arabinoside. Additive effects were recorded for combinations with prednisolone and VP-16-213, while DDP reduced the capacity of cells to absorb sublethal damage induced by alkylating agents.**

[Cancer Treat Rep 60:1619–1625, 1976]

Oncologists are presently looking for new approaches to systemic treatment for unresectable tumors. These approaches include the search for new, more effective antitumor agents and the utilization of combinations of drugs. The magnitude of such a search at the clinical level is hindered by the fact that it entails experimentation on human subjects with the consequent difficulties in standardization, reproducibility, and speed, its considerable expense, and the limitations in the number of compounds that can be evaluated. Cell cultures provide a rapid, efficient, and economic system for defining potentially useful combinations of chemotherapeutic drugs (1). Even though it is impossible to study every possible pair combination (2), we currently are investigating the combined effects of several drugs under study in our laboratory in an attempt to detect synergistic pairs. A drug is paired with other chemotherapeutic agents in order to observe whether the first drug can increase the killing effect of the second.

This paper reports results using *cis*-dichlorodiammineplatinum(II) (DDP) in combinations with 11 other antitumor agents. Although the antitumor effect of DDP was known since 1969 (3), its toxic effects (ie, nephrotoxicity, myelosuppression, ototoxicity, etc) precluded its extensive clinical utilization. Yet, its efficacy against a wide range of neoplasias (4) and newly devised means to ameliorate nephrotoxicity (5,6) have recently produced a renewed interest in this drug for the antitumor arsenal (7–11).

MATERIALS AND METHODS

Monolayer cultures of T_1 cells, a human lymphoma cell line (12), were maintained in Ham's F-10 medium supplemented with 20% fetal calf serum, glutamine, vitamins, and antibiotics. Cell survival was assayed by the colony-formation technique method as previously described (13,14). Drug solutions were freshly prepared before each experiment and appropriate concentrations were diluted in growth medium and applied in pairs (ie, DDP and BCNU; DDP and bleomycin, etc) to the cells for 1 hour at 37°C (pH 7.0–7.2). DDP was always used in a concentration of 10 μg/ml, which reduces the survival of T_1 cells by > 80%, while the other drugs were given in increasing concentrations. In every experiment, control survival curves were performed for each drug individually. Data were analyzed with

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TABLE 1.—Lethal effects of simultaneous treatment with DDP and other drugs*

Drug	Survival of T_1 cells treated with DDP alone (mean ± SE)	Concentration (μg/ml)	% survival with drug alone (mean ± SE)	% expected survival in combination (additive effect)	Observed survival in combination (% mean ± SE)	Significant deviation from predicted response†	Performance index
Methyl-CCNU	12.4 ± 2.6	10	93 ± 6.8	11.52	6.8 ± 1.43	S,$P < 0.01$	13.00
		25	52.6 ± 7.6	6.54	0.26 ± 0.05		
		50	4.6 ± 0.87	0.51	0.025 ± 0.0006		
		75	0.29 ± 0.07	0.035	—		
CCNU	15.6 ± 4.0	10	100 ± 8.8	15.36	9.68 ± 1.2	S,$P < 0.01$	2.38
		25	58.8 ± 3.0	9.2	3.28 ± 1.2		
		50	15.0 ± 1.5	2.34	0.22 ± 0.8		
		75	0.6 ± 0.1	0.094	—		
BCNU	19 ± 1.0	10	89.3 ± 11.5	17	8.1 ± 0.7	S,$P < 0.01$	8.68
		25	79 ± 6.9	15	3.2 ± 0.61		
		50	20 ± 1.2	4	0.3 ± 0.00		
		75	3 ± 0.17	0.56	—		
Yoshi 864	17 ± 2.2	10	100 ± 10	17	15.4 ± 1.5	S,$P < 0.01$	2.38
		25	92 ± 8	15	6.9 ± 0.96		
		50	54.1 ± 7.2	9	—		
		75	21 ± 2.3	3.6	1.9 ± 0.14		
		100	9.7 ± 0.83	1.6	1.1 ± 0.21		
Bleomycin	16 ± 0.82	10	31 ± 3.6	4.9	3.6 ± 0.53	S,$P < 0.01$	7.07
		25	15.2 ± 0.72	2.5	1.5 ± 0.08		
		50	4.4 ± 0.24	0.70	0.42 ± 0.04		
		100	3.8 ± 0.83	0.61	0.03 ± 0.01		
Cytosine arabinoside	32 ± 4.9	100	100 ± 9	33.4	32 ± 5.2	S,$P < 0.01$	2.11
		250	100 ± 3	49	22.3 ± 3.8		
		500	100 ± 12.5	35.1	15.4 ± 2.7		
		750	100 ± 12.1	34.1	11.6 ± 2.9		
Methotrexate	13 ± 1.2	50	91 ± 28	11.5	19.2 ± 2.3	A,$P < 0.01$	2.70
		100	68 ± 18	8.7	14 ± 1.7		
		500	42 ± 11	5.3	13 ± 1.4		
		1000	37 ± 4.3	4.7	24 ± 3.9		
Prednisolone	15 ± 0.7	10	100 ± 5.2	15	14 ± 7	NS	
		25	87 ± 11.5	13	24 ± 6		
		50	72 ± 3.4	10.8	6 ± 2		
		75	60 ± 2.7	9.0	8.8 ± 1.1		
		100	56 ± 9.8	8.4	—		
Adriamycin	19.8 ± 1.14	0.1	47 ± 1.1	9.3	0.79 ± 0.14	S,$P < 0.01$	8.58
		0.25	27 ± 2.2	5.3	1.8 ± 0.21		
		0.5	17 ± 1.8	3.4	1.04 ± 0.06		
		0.75	7.2 ± 0.52	1.4	0.09 ± 0.01		
		1.0	2.5 ± 0.28	0.50	0.06 ± 0.01		

Camptothecin	18 ± 4.3	5	75 ± 4.3	13.4	9.2 ± 0.8	S,$P < 0.01$	5.73
		10	62 ± 1.8	11.1	3.0 ± 0.3		
		25	49 ± 3.13	8.8	0.7 ± 0.01		
		50	50 ± 4.3	9.0	0.5 ± 0.03		
		75	52 ± 3.0	9.4	0.44 ± 0.03		
VP-16-213	23 ± 5.3	1	100 ± 7.4	23	20.1 ± 1.8	NS	
		2.5	47 ± 3.2	11.0	11 ± 0.7		
		5.0	34 ± 2.9	7.9	11.3 ± 1.7		
		7.5	12 ± 6.0	2.8	3.4 ± 2.4		

*Results from a single representative experiment for each combination.
†A = antagonistic; S = synergistic; NS = no significant deviation.

the aid of a CDC Cyber 73 digital computer and predicted additive effects were compared to the observed response. Each combination experiment was performed at least twice with three replicates per dose point and six replicate controls (untreated cells). Significant deviations from the additive response and their magnitude were defined as previously described (15). Briefly, for each dose point of the drug tested in combination with DDP, an additive response (% expected survival) was defined by the geometric sum of the survival resulting from treatment with each drug alone. An additive response for the tested drug was defined by a score obtained from the arithmetic sum of the results at each dose point. The variance of this score was determined by the technique of propagation of error (16). Deviations from the additive effect were considered significant when they exceeded $1.96 \times \sqrt{\text{variance}}$ (17). The overall significance of the deviation was accepted when the results were recomputed after eliminating the dose point with the greatest deviation. This procedure was instituted to avoid bias in the overall estimation introduced by a single dose point. In order to quantify deviations from additive effects, a performance index was devised. The performance index was obtained by averaging the sum of ratios of observed and expected survivals for each point. To calculate the ratios of synergistic drugs, the expected response was used as the denominator. The observed response was used as the denominator in antagonistic situations. This was done in order to obtain values > 1 so that the efficiency of antagonistic and synergistic drugs could be compared.

RESULTS

DDP was effective in increasing the killing efficiency of many different drugs when tested in two-pair combinations (table 1). True synergistic effects were noted for methyl-CCNU, BCNU, bleomycin, adriamycin, and camptothecin, where the combined killing effect was greater than threefold over the predicted additive effect. Greater than additive but less than threefold increases in lethality were measured for CCNU, Yoshi 864, and cytosine arabinoside. Additive results were obtained for the combinations of DDP with prednisolone or VP-16-213, while a subadditive effect was recorded for methotrexate. It is noteworthy that for all combinations with alkylating agents and nitrosourea derivatives, the main effect of DDP was to reduce the shoulder region of the survival curve (fig 1), ie, the capacity to absorb lethal damage (18) while the slopes of the exponential part of the curve, which reflects the sensitivity of the critical target, remained largely similar. Such profound changes in curve shape were absent for combinations involving other drugs tested in this study.

DISCUSSION

Renewed interest has recently been generated about the utilization of DDP as an antitumor agent. Although phase I and II clinical trials have been completed (4), the need arises for thorough knowledge about the properties of DDP in order to provide rational combinations for further studies. A considerable body of knowledge is presently available about the biomolecular mechanisms, the pharmacologic properties, and the cellular effects of DDP (19–29). This report supplements this knowledge by providing information on potentially useful combinations for clinical trials. DDP appears an effective complement for virtually every drug studied in this investigation since it produces synergistic or supra-additive effects. In this and in other investigations (15), we arbitrarily define true synergistic effects as those quantitatively greater by more than threefold from the predicted additive effect. Deviations from additive effects smaller than threefold are called supra- or sub-additive; true antagonistic effects are defined by cellular killing less than that obtained by the most powerful drug of the pair alone. Contrary to our previous report (15) showing simple additivity for the combination of adriamycin and DDP, the present investigations showed a significant synergistic effect for this two-drug combination (performance index, 8.6). It is possible that such results are due to different ratios of the drugs used and that synergistic effects can be appreciated better by pairing drugs in multiple ratios (30). It is noteworthy that contrary to the data obtained in the L1210 leukemia system (31), both camptothecin and BCNU appeared clearly synergistic with DDP in human cultured cells. Differences may be related to species response, pharmacologic properties, or scheduling variations. In fact, DDP also increased the lethality of two other nitrosoureas, methyl-CCNU and CCNU, and that of the alkylating agent, Yoshi 864. The survival curves of all of these agents showed one common phenomenon—the shoulder region was either cancelled or maximally reduced by DDP. This indicates that the latter drug affects the capacity of cells to absorb lethal damage, possibly by modifying the structure of the target sites. Crosslinking by DDP (32) could provide a rigid DNA structure more susceptible to the attack of the free radicals

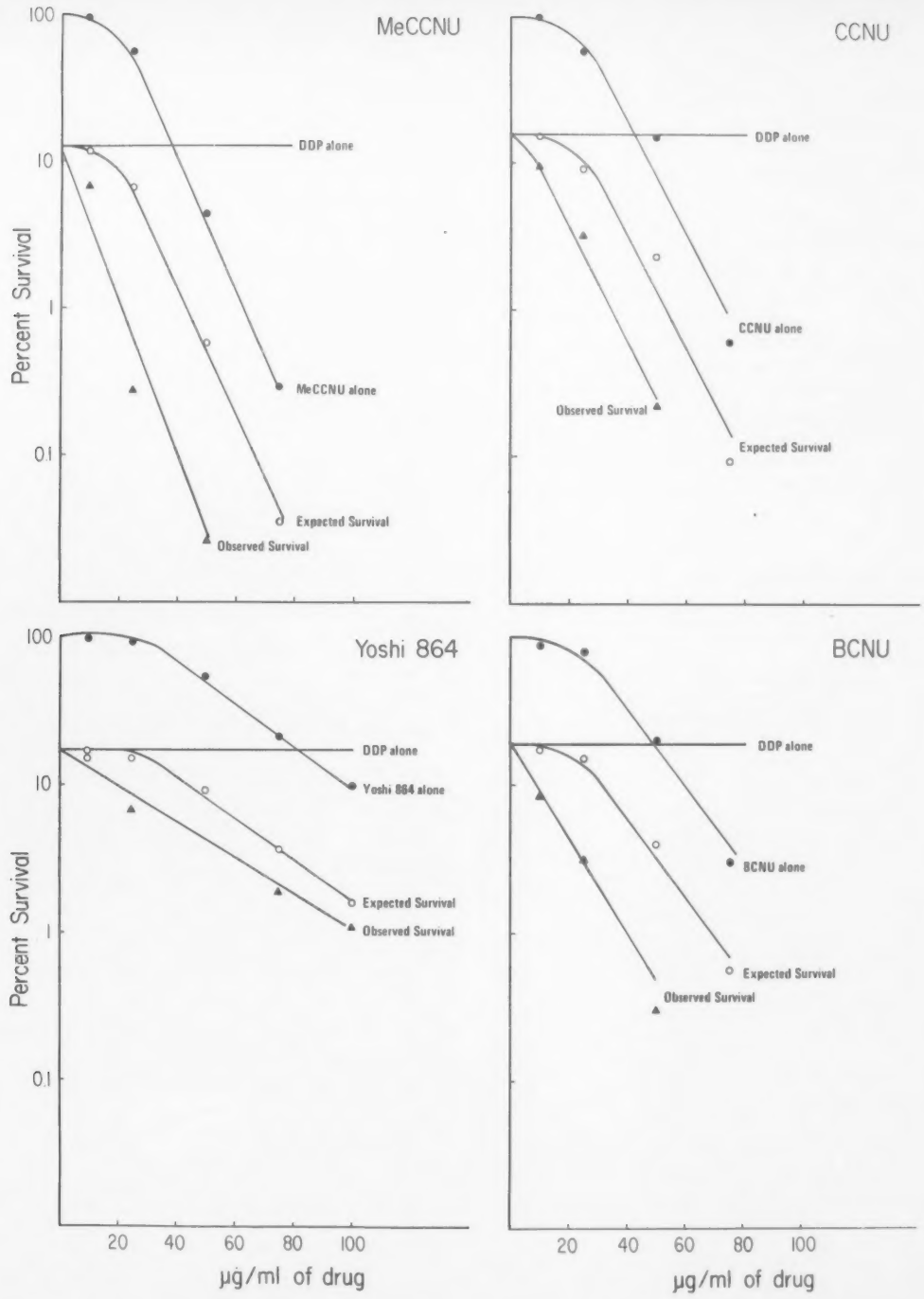

FIGURE 1.—Survival curves demonstrating synergistic killing effects of DDP (10 μg/ml) used in simultaneous pair-combination treatment with increasing concentrations of alkylating agents and nitrosourea derivatives.

of an alkylating agent such as Yoshi 864 or to sustained crosslinking by the nitrosoureas (33). A tenfold increment in lethal effects with DDP and camptothecin over that expected from the summation of the effects with each drug alone was noted. It is not apparent how DDP and camptothecin exert their combined effect. It is possible that platinum-induced crosslinking of DNA, or its effect on template inactivation (24), may facilitate the mode of action of camptothecin which acts either by inhibiting DNA synthesis by a direct effect on the DNA template (34,35) or by producing strand breaks (36). Similar speculations (37,38) apply for the considerable synergistic effect noted for the DDP-bleomycin pair. From the data presented in this report, it follows that increased therapeutic efficacy can be expected by combining DDP with a variety of presently used antitumor agents and that its inclusion in treatment strategies should enhance the current resources of antineoplastic drug combinations.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8
BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
Bleomycin: NSC-125066; CAS reg. No. 11056-06-7; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate
Camptothecin: NSC-100880; CAS reg. No. 25387-67-1
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
***Cis*-dichlorodiammineplatinum(II):** NSC-119875; CAS reg. No. 15663-27-1
Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-
Prednisolone: NSC-9900; CAS reg. No. 50-24-8; pregna-1,4-diene-3,20-dione, 11β,17,21-trihydroxy-
VP-16-213: NSC-141540; 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside)
Yoshi 864: NSC-102627; CAS reg. No. 3458-22-8; 1-propanol, 3,3′-iminodi-, dimethanesulfonate (ester), hydrochloride

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Distribution of Tetrahydrouridine in Experimental Animals [1,2,3]

Salah M. El Dareer,* Vivian White, Francis P. Chen, L. Bruce Mellett, and Donald L. Hill [4,5]

SUMMARY

2-^{14}C-tetrahydrouridine was prepared and used to determine the serum levels and excretion of tetrahydrouridine by various experimental animals. In mice injected ip with the agent (50 mg/kg), the serum level of tetrahydrouridine was maximum (76 μg/ml) at 15 minutes. For rats injected with the same dose, the tetrahydrouridine content of serum was greatest (58 μg/ml) at 30 minutes. Within 3 hours, the serum content of the drug in both mice and rats fell to < 10% of the maximum. The kidneys of mice selectively accumulated tetrahydrouridine; the concentration rose to 275 μg/g at 1 hour after injection. Nearly all of the dose was excreted unchanged in the urine of mice and rats in 24 hours. For a dog and a monkey given an iv dose (50 mg/kg) of tetrahydrouridine, serum levels of the agent were 210 and 200 μg/ml, respectively, at 5 minutes. The apparent half-lives for the initial phase of disappearance were 18 and 20 minutes and those for the later phase were 65 and 70 minutes, respectively. In 24 hours the dog and monkey excreted most of the dose as unchanged tetrahydrouridine. No metabolites were detected in the biologic samples from either species.

[Cancer Treat Rep 60:1627–1631, 1976]

Cytosine arabinoside (araC)[6] has activity against a variety of rodent and human neoplasms (1,2). Primarily due to its deamination to 1-β-D-arabinofuranosyluracil, an inactive compound, araC rapidly disappears from the blood of cancer patients (3,4). Tetrahydrouridine, a transition-state analog (5), is a potent inhibitor of the deaminase that catalyzes removal of the amino groups on araC and various analogs of cytidine and 2′-deoxycytidine (6–11). Studies in mice, dogs, and monkeys show that the inhibitor, when given concomitantly with araC, raises and prolongs the serum levels of this agent (7,12). Tetrahydrouridine also enhances the oral activity of 5-azacytidine against L1210 leukemia in mice (13). Administered alone, tetrahydrouridine causes no notable toxic effects (14). Tetrahydrouridine increases the incorporation of 2′-deoxycytidine into DNA of several types of tumor cells (15) and increases araCTP synthesis in human leukemic cells (16). Although the combination of araC and tetrahydrouridine has been tested against several tumors, an increased therapeutic index has been established only for the ascitic form of Sarcoma 180 (17).

The present study was undertaken to determine the serum and tissue levels and excretion patterns for tetrahydrouridine in experimental animals. A preliminary report of this work has been presented (18).

MATERIALS AND METHODS

Bio-Solv BBS-3 solubilizer was purchased from Beckman Instruments (Palo Alto, Calif) and Aquasol and Liquifluor scintillation solutions were purchased from New England Nuclear Corp (Boston, Mass). Chrom AR 1000 sheets were purchased from

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[2]Supported by contract N01-CM-43780 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Tetrahydrouridine: NSC-112907; CAS reg. No. 5627-05-4; 2(1*H*)-pyrimidinone, tetrahydro-4-hydroxy-1-β-D-ribofuranosyl-. Supplied by the Developmental Therapeutics Program, DCT, NCI.

[4]Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Ala.

[5]We are grateful to Mr. Marion Kirk for performing the mass spectral analyses.

[6]Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; araC.

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Mallinckrodt (St. Louis, Mo) and silica gel thin-layer sheets, type SA, from Gelman Instrument Co (Ann Arbor, Mich). All radioactive samples were counted in a liquid scintillation spectrometer equipped with an external standard. The counting efficiency was 79%.

In the preparation of labeled tetrahydrouridine, an aqueous suspension (79 ml) containing 565 microcuries of 2-^{14}C-cytidine (25 millicuries/mmol; Schwarz/Mann Biochemicals, Orangeburg, NY), 500 mg of unlabeled cytidine, and 0.1 g of 5% rhodium on carbon catalyst (Engelhard Industries, Inc, Newark, NJ) was reduced in a Parr hydrogenation apparatus at 2.4 atmospheres of hydrogen at room temperature for 24 hours (19). As determined by thin-layer chromatography on Chrom AR 1000 strips with chloroform:methanol (3:1, vol/vol) as the solvent and scanning of the strips with a Packard 2100 radiochromatogram scanner, the preparation contained three compounds (R_f = 0.02, 0.21, 0.55), one of which was tetrahydrouridine (R_f = 0.55). The presence of the target compound was established by its migration on thin-layer chromatography and by analysis with a Hitachi mass spectrometer (model RMU-6-D). Tetrahydrouridine has peaks (with relative intensities) at m/e 230 (2), 127 (45), 97 (80), 56 (95), and 28 (100). The value of 230 corresponds to the molecular weight minus a molecule of water. It was purified by twice-repeated thin-layer chromatography on Chrom AR 1000 sheets. For the final chromatogram, the portion containing tetrahydrouridine was cut from the sheet and the compound was eluted with water. After filtration to remove the silica gel, the solution was evaporated to yield solids with a specific activity of 0.4 microcurie/mg. The yield was 15%. The radiochemical purity of the preparation was 95%, as judged by further thin-layer chromatography in a solvent of chloroform:methanol (1:1, vol/vol). (The R_f for tetrahydrouridine was 0.43.) No distinct impurity was present. Only a single radioactive spot was detected on paper chromatograms developed in 1-butanol:water (86:14, vol/vol) (R_f = 0.10) or 2-propanol:concentrated ammonia:water (7:1:2, vol/vol/vol) (R_f = 0.53) and scanned with the radiochromatographic scanner.

The animals used were male BDF$_1$ mice weighing 20–25 g, male Sprague-Dawley rats weighing 250–300 g, a female beagle dog weighing 10 kg, and a female rhesus monkey weighing 4.1 kg. For all animals, tetrahydrouridine (50 mg/kg) was injected as a solution in 0.9% sodium chloride. This dose, administered 30 minutes before araC, strongly inhibits the deamination of araC in tissues of several animal species (S. M. El Dareer and L. B. Mellett, unpublished results). Unlabeled tetrahydrouridine was added so that the injected solutions contained drug of the following specific activities: for mice, 0.32 microcurie/mg; for rats, 0.093 microcurie/mg; for the dog, 0.029 microcurie/mg; and for the monkey, 0.046 microcurie/mg. All animals were starved overnight before being treated. Urine was collected by catheters. Blood was collected from the axillary region of mice anesthetized with carbon dioxide shortly before collection and from the anterior radial or saphenous veins of the monkey and dog. Organs from mice were collected, placed on solid carbon dioxide, pooled separately, and homogenized in 3 volumes of water with a Brinkman Polytron equipped with a P-10 generating head (Brinkman Instruments, Westbury, NY). For determination of total radioactivity in biologic samples, 15 ml of Liquifluor containing Bio-Solv BBS-3 (14:1, vol/vol) was added to a 0.1-ml portion prior to scintillation counting. Extracts of serum and various homogenized tissues were prepared by addition of 3 volumes of ethanol and centrifugation at 2000 × g. This procedure allowed recovery of 96%–103% of the radioactivity present. Urine samples were spotted directly onto thin-layer sheets. Chromatography was accomplished on thin-layer sheets, type SA, in chloroform:methanol (3:1, vol/vol). Radioactivity on the developed sheets was measured by cutting 0.5-cm strips, placing them in vials containing 1 ml of water, and subjecting the vials, with Aquasol solution added, to scintillation counting. The R_f for tetrahydrouridine on these sheets was 0.65.

RESULTS AND DISCUSSION

For mice injected ip with 50 mg/kg of ^{14}C-tetrahydrouridine, a peak serum level of 76 μg/ml was observed at 15 minutes (fig 1). The level then declined with a half-life of 40 minutes. Tissue levels of tetrahydrouridine in mice at various times after treatment are shown in figure 2. After 5 minutes, the concentrations present in liver, washed intestine, and spleen were similar to those in serum. The concentration in the skeletal muscle did not reach this level, and < 1.5 μg/g was present in the brain at any time. Accumulation of tetrahydrouridine in the kidney throughout 1 hour is noteworthy. In 24 hours eight mice excreted 102% ± 6% of a 100-mg/kg dose of tetrahydrouridine in the urine as intact drug—a finding in accord with the accumulation of tetrahydrouridine in the kidneys.

In rats injected ip with 50 mg/kg of ^{14}C-tetrahydrouridine, a peak serum level of 58 μg/g was observed at 30 minutes (fig 1). For the initial phase of disappearance, the half-life was about 15 minutes. In 24 hours, two rats excreted 91% and 99% of the total dose in the urine.

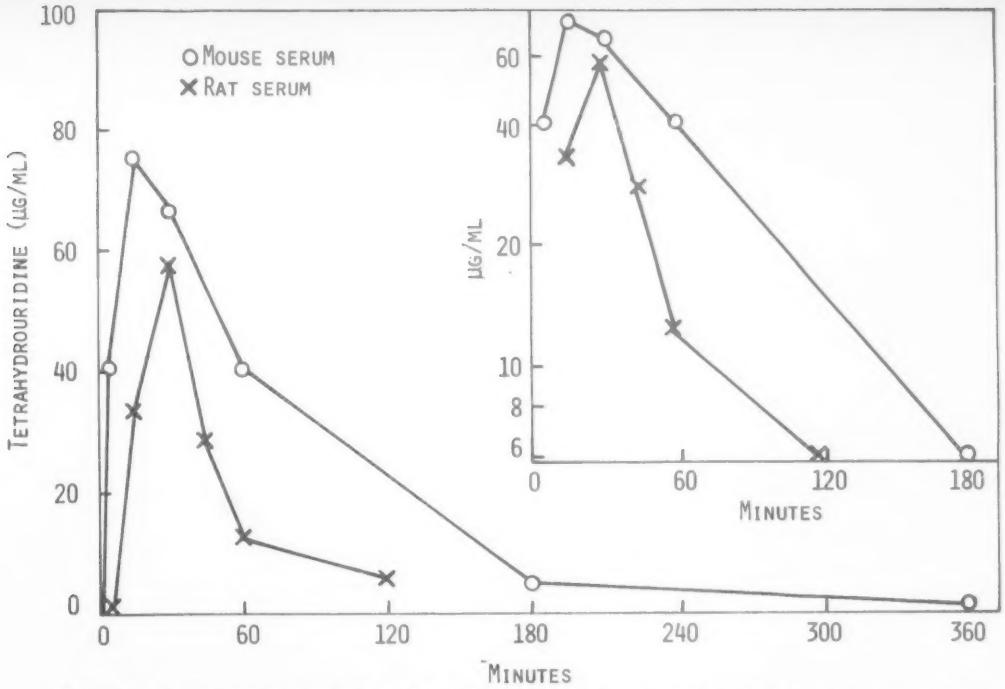

FIGURE 1.—Serum levels of tetrahydrouridine in mice and rats injected ip with 50 mg/kg. Each time point represents pooled serum from 5 mice or 3 rats. Values for the vertical axis of the inset figure are on a log scale.

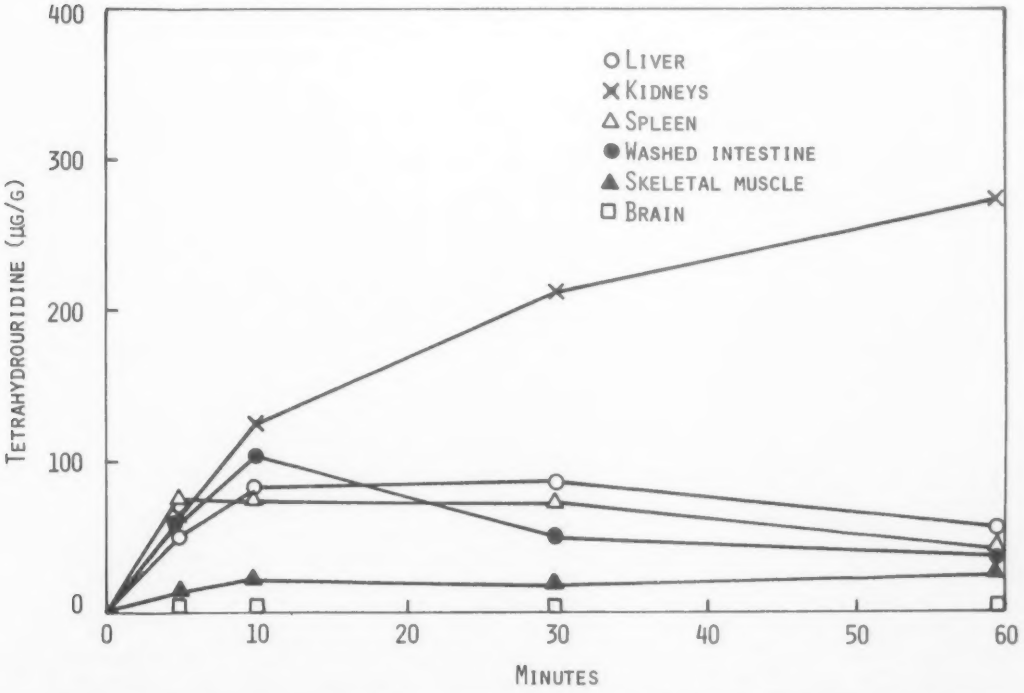

FIGURE 2.—Tissue levels of tetrahydrouridine in mice injected ip with 50 mg/kg. Each point represents the value for pooled tissues from 5 mice. The gastrocnemius muscle was assayed.

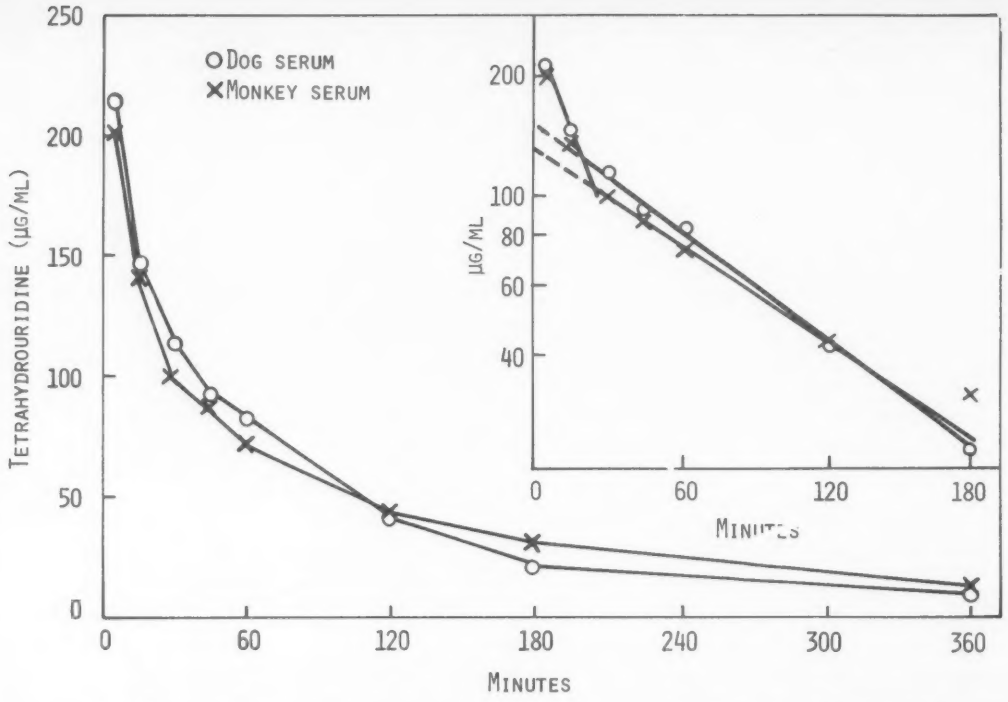

FIGURE 3.—Serum levels of tetrahydrouridine in a dog and a monkey after iv injection of 50 mg/kg. Values for the vertical axis of the inset figure are on a log scale.

The disappearance of tetrahydrouridine from the serum of a dog injected iv with a dose of 50 mg/kg is illustrated in figure 3. The half-life for the initial phase was 18 minutes and that for the later phase was 65 minutes. In 24 hours, 84% of the administered dose was excreted in the urine, all as unchanged drug. For a monkey injected with the same dose (fig 3), the half-life for the initial phase was 20 minutes, and that for the later phase was 70 minutes. In 24 hours, 70% of the dose was excreted in the urine as tetrahydrouridine. During this time, < 0.3% of the dose was excreted in the feces.

No metabolites of tetrahydrouridine were detected in any of the biologic samples. As judged from thin-layer chromatograms with several solvents, the largest amount of metabolites that could be present in any sample was < 5% of the total radioactivity in that sample.

The rapid excretion of tetrahydrouridine by all four species indicates that, to be most effective, the agent should be administered shortly before the serum araC concentration drops below the minimum therapeutic level. After the concentration of tetrahydrouridine in serum and tissues has dropped to < 1 µg/ml (1 µg/g)—the minimum amount providing essentially complete in vitro inhibition of deamination of araC by the deaminases in human liver and granulocytes (7,20,21)—there is not likely to be useful inhibition of the degradation of araC in intact experimental animals or man.

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Phase I Study of Oral Mitomycin C[1,2]

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SUMMARY

This study demonstrates that the gastrointestinal absorption of oral mitomycin C is variable and that myelosuppression correlates most closely with the peak serum concentration. The probable maximal-tolerated dose of oral mitomycin C is 45–50 mg/m^2.

[Cancer Treat Rep 60:1633–1636, 1976]

Mitomycin C is an alkylating agent active against certain adenocarcinomas (1,2). Recently, intermittent dose schedules have resulted in enhanced activity and more manageable toxic effects (2). The most significant toxic effect associated with mitomycin C is delayed cumulative myelosuppression (2,3). An additional toxic manifestation is severe cellulitis occurring if extravasation occurs during iv administration. To allow improved ease of administration and to avoid the possibility of extravasation-associated cellulitis, oral administration is a potential alternate method of mitomycin C administration. Although oral mitomycin C is currently utilized in Japan and Europe, no phase I data have been reported. Consequently, we undertook a phase I study of oral mitomycin C.

Animal studies have shown that mitomycin C is absorbed after oral administration. However, the oral LD50 was three to 12 times the iv LD50 (4,5). Oral absorption in animals was reported to be enhanced by the concomitant oral administration of $NaHCO_3$ (5).

MATERIALS AND METHODS

Mitomycin C was supplied in No. 4 hard gelatin capsules containing 0.5 or 2.5 mg of mitomycin C. Sodium bicarbonate was supplied as compressed tablets containing 0.32 g of $NaHCO_3$. These mitomycin C capsules were refrigerated until used.

Patients who had biopsy-proven carcinoma, sarcoma, leukemia, or lymphoma with clear evidence of metastatic involvement were admitted to the study if they were not candidates for known regimens of higher potential benefit. All patients were required to have a life expectancy of $\geq$ 12 weeks and a Karnofsky performance status of $\geq$ 30%.

Patients were admitted to the study if they had received no other chemotherapy for 4 weeks previously and if they had not received iv mitomycin C or porfiromycin. Absolute polymorphonuclear neutrophil and platelet counts > 2000 cells/mm^3 and 100,000 platelets/mm^3, respectively, were required. Serum BUN and creatinine levels < 20 mg/100 ml and 1.2 mg/100 ml, respectively, were required. A total of 20 men and 11 women were entered into the study. The mean age was 59 years. Diagnoses included lung cancer (20 patients), adenocarcinoma of the breast (five patients), gastrointestinal tract (three patients), and miscellaneous tumors (three patients). Sixteen of the patients had received prior chemotherapy. Fourteen patients had received prior radiotherapy; two of these had had extensive radiotherapy to marrow-bearing areas.

Patients were allowed nothing orally starting at 12 AM of the treatment day. Eighteen of the 31 patients received 3.2 g of oral $NaHCO_3$ with 120 ml of water 15 minutes prior to administration of mitomycin C. Patients were given nothing further orally until 12 PM of the treatment day. All vomitus was saved, and the concentration of mitomycin C in the vomitus was determined.

Blood was drawn at 0, 5, 15, 30, 60, 120, 180, and 300 minutes after a dose of mitomycin C. Serum,

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[2]Mitomycin C: NSC-26980; CAS reg. No. 50-07-7.

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TABLE 1.—Patient evaluability

Mitomycin C dose (mg/m^2)	Patients evaluable for—*		
	Efficacy	Toxicity	Pharmacokinetics
5.0	2/3	3/3	3/3
7.5	3/3	3/3	3/3
10.0	3/4	3/4	3/4
12.5	1/1	1/1	1/1
15.0	2/4	3/4	4/4
18.0	1/1	1/1	0/1
22.5	2/2	2/2	2/2
30.0	6/8	7/8	6/8
45.0	4/5	4/5	2/5
Totals	24/31	27/31	24/31

*No. of evaluable patients/total No. of patients treated.

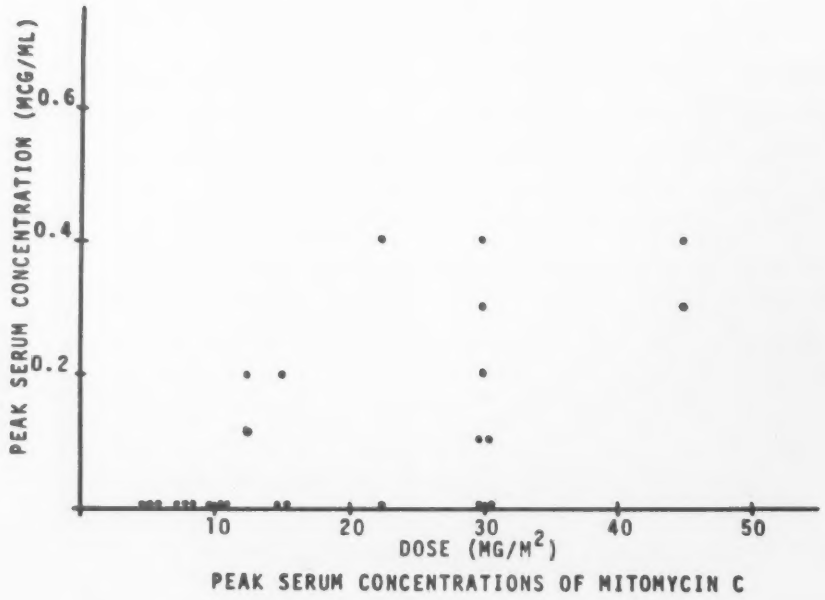

FIGURE 1.—Maximum serum concentration of mitomycin C in patients receiving oral mitomycin C. The assay was performed as described in Materials and Methods. Each point represents the peak serum concentration obtained in a patient after a dose of mitomycin C.

urine, and vomitus were frozen immediately after collection. The assays were performed at Bristol Laboratories as previously described (6).

RESULTS

Of the 31 patients enrolled in the study, 27 were evaluable for toxicity and 24 were evaluable for efficacy and pharmacokinetics. The doses employed and information on patient evaluability are shown in table 1. Eight patients were treated with two courses of mitomycin C, three patients with three courses, and one patient with five. Death occurred in 11 patients prior to a second course, and in seven patients progression of disease made a second course therapeutically unattractive.

Pharmacokinetics and Toxicology

Eleven of 24 patients evaluable for pharmacokinetics had measurable serum mitomycin C concentrations. Figure 1 shows the peak serum concentra-

TABLE 2.—Toxic effects

Mitomycin C dose (mg/m^2)	No. of evaluable patients	No. of patients with—			
		Nausea and vomiting	Diarrhea	Leukopenia	Thrombocytopenia
5	3	0	0	0	0
7.5	3	0	0	0	0
10	3	0	0	0	0
12.5	1	0	0	0	0
15	3	2	0	0	0
18	1	0	0	0	0
22.5	2	1	0	0	1
30	7	1	0	1	0
45	4	3	1	2	2

TABLE 3.—Patients experiencing myelosuppression after their first dose of mitomycin C

Mitomycin C dose (mg/m^2)	Patient No.	Nadir ($\times 10^3$)	Time to nadir (days)	Peak serum concentration (mg/ml)	Previous chemotherapy	Complications
Leukopenia (wbcs < 4000/mm³)						
30	1	2.6	51	0.4	5-FU, cyclophosphamide	None
45	2	3.5	20	Unevaluable	None	None
45	3	3.7	16	0.4	None	None
Thrombocytopenia (platelets < 100,000/mm³)						
22.5	4	54	12	0.4	Cooper, adriamycin*	None
45	5	27	16	0.4	None	None
45	6	72	33	Unevaluable	None	None

*This patient was treated initially with the Cooper regimen, relapsed, and was then treated with adriamycin.

tions noted in each patient. The maximum peak serum concentration was 0.4 μg/ml. Serial serum determinations demonstrated that the shape of the serum concentration curves also varied. Ten of the 24 evaluable patients had detectable mitomycin C concentrations in the urine after 6 hours, accounting for 0–4.4% of a total dose.

The toxic effects experienced are shown in table 2. In general, the incidence of toxic effects increased with increasing doses. Gastrointestinal toxicity was not a major problem. No patients experienced alopecia.

Characteristics of patients who experienced myelosuppression after one dose of mitomycin C are shown in table 3. The incidence of myelosuppression correlated well with the peak serum concentrations, poorly with the dose, and not at all with the incidence and type of previous chemotherapy. None of the patients who experienced myelosuppression had evidence of compromised hematopoiesis at the start of the therapy. At a dose of 45 mg/m^2, one possible drug-related death was noted in a patient who had myelosuppression. However, this patient experienced leukopenia within 10 days of mitomycin C administration and died. The role of mitomycin C in the development of leukopenia in this patient is unclear. Evidence of cumulative marrow toxicity was not detected at the doses studied in any of the patients treated with more than one course of mitomycin C. In no patient was there a $> 25\%$ increase in BUN or creatinine during this study. Neither of the patients who had received prior extensive radiotherapy experienced any toxicity due to mitomycin C.

The simultaneous administration of oral $NaHCO_3$ administered to 18 patients had no demonstrable effect on the absorption of mitomycin C or on its toxic effects.

Therapeutic Response

Twenty-four patients were evaluable for mitomycin C efficacy. No complete or partial responses were noted. One patient experienced stable disease.

DISCUSSION

This study demonstrates (*a*) that gastrointestinal absorption of mitomycin C is variable, (*b*) that the toxic effects of oral mitomycin C are similar to those of parenteral mitomycin C and that the myelosuppression correlates most closely with peak serum concentrations, (*c*) that concomitant administration of $NaHCO_3$ has no demonstrable effects on absorption or toxic effects, and (*d*) that the probable maximal-tolerated dose is 45–50 mg/m^2 every 8 weeks. (Treatment of several patients at doses > 45 mg/m^2 would be necessary to determine with precision the maximal-tolerated dose.)

The low percentage of mitomycin C found in the urine indicates that, in a manner similar to iv administered mitomycin C, serum clearance of orally administered mitomycin C is effected primarily by means other than renal excretion.

It is not surprising that no therapeutic responses were observed since many of the patients who were treated had tumors generally considered unresponsive to mitomycin C and most were treated with clearly subthreshold doses.

Since mitomycin C is a highly toxic drug, and this study demonstrates variable gastrointestinal absorption, the oral administration of mitomycin C can not be recommended.

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Survival and Cycle-Progression Delay of Cultured Human Lymphoma Cells Treated With 1-Propanol, 3,3′-Iminodi-, Dimethanesulfonate (Ester), Hydrochloride (Yoshi 864) [1,2,3]

Benjamin Drewinko* and Barthel Barlogie [4,5]

SUMMARY

Asynchronous human lymphoma cells treated for 1 hour with increasing concentrations of 1-propanol, 3,3′-iminodi-, dimethanesulfonate (ester), hydrochloride (Yoshi 864) revealed a shouldered survival curve typical of the effects of alkylating agents and of ionizing radiation on this cell line. Yoshi 864 was unstable under the conditions of treatment, its killing effect being reduced by 50% after only 4 hours. Synchronized cells showed stage-dependent sensitivity: early-S, late-G_2, and late-G_1 phases were the most sensitive while mid- and late-S and early-G_2 phases were relatively insensitive. Yoshi 864 induced a concentration- and incubation time-dependent delay in the transit of asynchronous cells through G_2 phase, with maximum accumulation values obtained after 12 hours of incubation with 100 μg/ml. This effect was largely reversible and no further kinetic changes were noted in the progeny of treated cells. Incubation of synchronized cells for 1 hour with 100 μg/ml demonstrated a block in G_2, the manifestation of which during the lifespan of the treated cell or in its immediate progeny was cell-cycle dependent. Thus, cells treated in G_1, early-, and mid-S phases showed a delay in the subsequent G_2 phase while cells treated in late S and in G_2 manifested this effect in the G_2 phase of the immediate progeny. There was no correlation between this blocking effect in G_2 with cell survival assessed by colony formation. Yoshi 864, although a rather inefficient killing drug, may represent a useful chemical synchronizing agent.

[Cancer Treat Rep 60:1637–1645, 1976]

1-Propanol, 3,3′-iminodi-, dimethanesulfonate (ester), hydrochloride (Yoshi 864) is a new synthetic alkylating agent structurally similar to busulfan (1) (fig 1) presently undergoing clinical trials (2). Chemically, the drug is an aminoglycol methane-sulfonic acid ester derivative synthesized by Sakurai and El-Merzabani (3). Yoshi 864 is effective in various experimental tumor systems (4,5) where it does not show cross resistance to mechlorethamine (6). The oral form has been found to be active in human chronic granulocytic leukemia and lymphoma (1,7). This paper describes the lethal and cytokinetic effects induced by Yoshi 864 on a long-term culture of human lymphoma cells. Dose- and time-dependent responses are analyzed and cell cycle-related effects are reported. Although in comparison to other alkylating antitumor agents Yoshi 864 is a rather inefficient cell-killing agent, it appears to be an excellent candidate for synchronizing manipulations.

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[2]Supported by contracts N01-CM-53773 and N01-CM-43801 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Yoshi 864 (NSC-102627; CAS reg. No. 3458-22-8) was supplied by the DCT, NCI, as a dry powder and manufactured by the Ben Venue Laboratories, Bedford, Ohio. Dilutions in growth medium were made fresh before each experiment.

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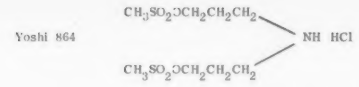

Busulfan $CH_3SO_2OCH_2(CH_2)_2CH_2OSO_2CH_3$

FIGURE 1.—Chemical structures of Yoshi 864 and busulfan.

MATERIALS AND METHODS

Cell Line

A human immunoglobulin-producing cell line (T_1 cells) derived from the tumorous lymph node of a patient with lymphocytic lymphoma was used (8). The cells carry a tumor-associated antigen common to lymphomas (9). Monolayer cultures were maintained in Ham's F-10 medium supplemented with 20% fetal calf serum, vitamins, glutamine, and antibiotics. Under these conditions, the cells grow with an average cell cycle time of 31 hours as determined by the pulse labeled mitosis (PLM) technique. The pre-DNA synthesis period (G_1) is 15 hours, the DNA synthesis period (S) is 10 hours, and the post-DNA synthesis period (G_2) is 6 hours (10).

Cell Survival

To assess survival, we harvested treated and control cells growing in 60-mm petri dishes by sequential enzymatic digestion with viokase (Grand Island Biological Co, Great Neck, NY) and trypsin (2.5% in Hanks' balanced salt solution) and counted them with the aid of an electronic particle counter (Coulter Electronics, Inc, Hialeah, Fla). Known aliquots were dispensed into 60-mm petri dishes so that 50–100 colonies would appear after 21 days of incubation in a 5% CO_2 humidified atmosphere at 37°C. The colonies were stained with 0.5% crystal violet in 95% ethanol. Viability was defined as the ability of single cells to give rise to a colony of ≥ 50 cells. In each experiment, at least six plating-efficiency controls were run in parallel. The survival fractions of the dose points were normalized with respect to the individual controls for each experiment. Control cells underwent all manipulations undergone by test cells but did not receive the drug. In this series of experiments, the plating efficiency ranged up to 40%. All experiments were done at least in duplicate with triplicate samples per dose or time point. Means, standard errors, and ancillary statistical analysis were calculated with the aid of a Cyber 73 computer (Control Data Corp, Minneapolis, Minn).

Cell Synchronization

Synchronized S-phase cells were obtained by a single treatment with 3 mM thymidine (TdR) for a 24-hour duration. As shown in previous studies (11), an adequate degree of synchrony is obtained by these procedures on T_1 cells. This synchrony extends for the length of at least one cell cycle. For the present experiments, the degree of synchrony was monitored in replicate cultures by 30-minute pulse labeling with 0.5 microcurie of [^{3}H]cytidine (specific activity 5 curies/mmol) to determine the percentage of cells in S phase and by scoring the mitotic index (MI), and by DNA histograms obtained by pulse cytophotometry as previously described (11). In all radioautography procedures, a 50% solution of Ilford K5 emulsion (Polysciences, Inc, Warrington, Pa) in distilled water was used. Labeled cells were identified by the presence of five or more grains overlying the nucleus. At the end of the synchrony procedure, 90% of the cells were in S phase and moved synchronously into G_2 phase (80%). After a mitotic peak of 8%–12% (the MI of asynchronous T_1 cells ranges from 0.6 to 1.8), the cells were still partially synchronized in G_1 phase (70%–80%). These procedures did not significantly alter the plating efficiency of T_1 cells, although they produced a shortening of S and G_2 phases and an elongation of G_1 phase.

Dose-Response Survival of Asynchronous Populations

Stock cultures of T_1 cells were harvested and cell suspension aliquots were seeded into 60-mm petri dishes (0.5×10^6 cells/dish). The cells were incubated at 37°C in a 5% CO_2 atmosphere in air for about 48 hours to achieve exponential growth. The medium was discarded and the cells were exposed to increasing drug concentrations for exactly 1 hour at 37°C. The drug was decanted and the cells were washed twice with Hanks' balanced salt solution. Colony-forming ability was assayed as described above.

Kinetics of Cell Kill

Cell survival as a function of duration of treatment was investigated by exposing replicate cul-

tures simultaneously to a single dose of drug. Petri dishes containing 0.5×10^6 cells in asynchronous exponential growth were incubated at 0 time with a single concentration of drug (50 μg/ml). Replicate cultures were harvested at regular intervals for 48 hours and survival was assessed as described above. Plating-efficiency controls were obtained at regular intervals. To demonstrate the stability of the biologic effect of the drug, a separate control system was instituted. This consisted of dishes also containing 0.5×10^6 cells which were kept growing without adding drug throughout the experiment. Every time a time-point dish was harvested, the supernatant medium of the control dish was discarded and the drug was decanted from the treated dish to the control dish. The latter was then incubated for 1 hour at 37°C and survival assessed as usual.

Cell Cycle-Dependent Survival

Stage-dependent survival was investigated by incubating synchronized cells with increasing concentrations of drug at regular intervals throughout the cell cycle. Plating-efficiency controls were obtained at each time point on cells which underwent all synchronization procedures and mechanical manipulations but were not exposed to drug.

Cell Cycle Progression

Cell cycle progression was monitored by sequential DNA histograms obtained by pulse cytophotometry. Techniques and methods of evaluation have been previously described (11). Briefly, ethanol-fixed cells were stained with mithramycin (50 μg/ml) containing an equimolar concentration of $MgCl_2$ (7.5 mM), and 5×10^4 to 10^5 cells were measured for each DNA histogram. Aliquots were also labeled with [^{3}H]TdR (1 microcurie/ml; specific activity 3.0 curies/mmol) and processed for radioautography as described above in order to define the labeling index (LI). The MI was determined by scoring 3000 cells.

Pulse cytophotometry studies were performed on asynchronous cells exposed to various concentrations of Yoshi 864 for different time periods. Duplicate samples were harvested both during drug exposure and after drug removal. Synchronized cells were exposed to the drug for 1 hour at regular intervals throughout the cell cycle. Duplicate samples of cells treated in the different stages were examined for periods ranging up to 200 hours after release of the TdR block. Standard deviations for each cell cycle compartment did not exceed 2.5%.

RESULTS

Cell Survival

The survival curve of asynchronous exponentially growing T_1 cells exposed to increasing concentrations of Yoshi 864 is shown on figure 2. The curve is a "C" type curve with a relatively small shoulder (quasi-threshold dose [D_q] = 12 μg/ml, 1 hour) followed by a logarithmic decrease in survival as a function of increasing concentrations. A D_0 (defined as the mean lethal dose necessary to reduce survivors to 37%) of 48 μg/ml (1 hour) indicates the

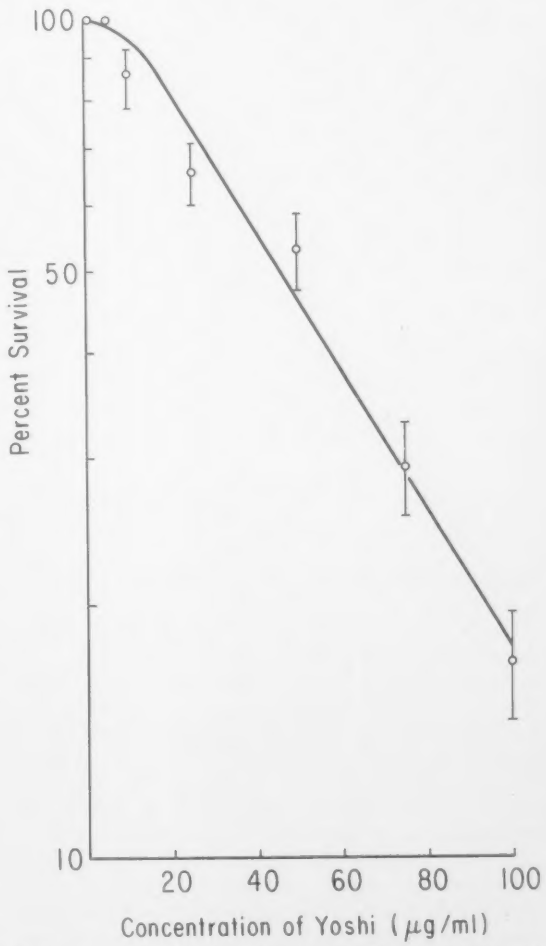

FIGURE 2.—Survival of asynchronous T_1 cells as a function of increasing concentrations of Yoshi 864 incubated for 1 hr. Data points are the mean value of 7 separate experiments with 3 replicates/dose point each. SEs are represented by the bars unless smaller than data points.

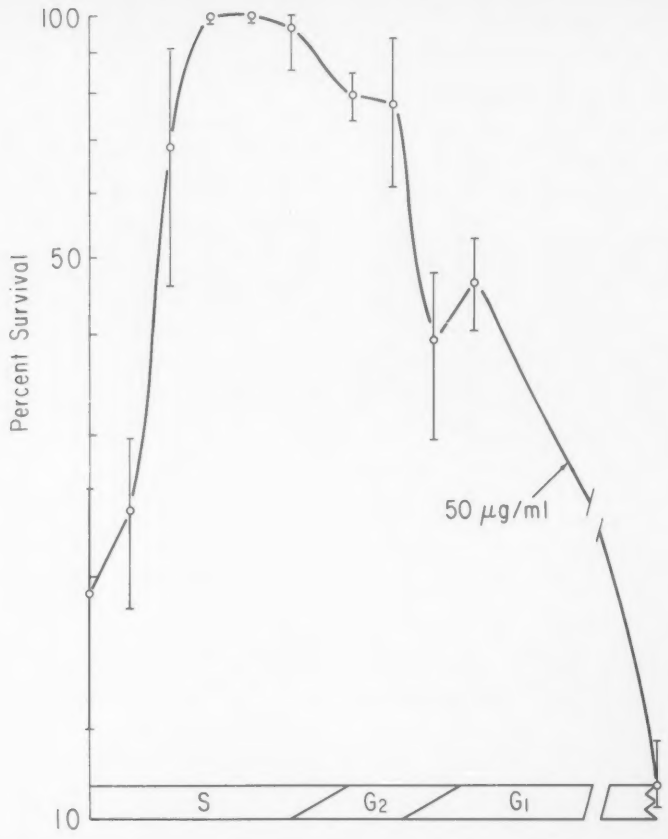

FIGURE 3.—Effect of a single concentration of Yoshi 864 (50 μg/ml) incubated for 1 hr with synchronized T_1 cells at different points of the cell cycle. Bars represent SEs.

relatively poor efficiency of Yoshi 864 in decreasing cell survival.

Yoshi 864 was very unstable in growth medium. After 4 hours of continuous incubation with T_1 cells, the decanted drug solution was only 50% as effective in reducing the survival of control cells incubated for 1 hour in comparison to cells treated for 1 hour with the freshly prepared drug. After 24 hours of continuous incubation, no killing effect was elicited on control cells incubated for 1 hour with the decanted drug solution.

Synchronized cells showed an evident cell cycle-dependent response to Yoshi 864 (fig 3). Early-S phase cells were relatively sensitive while late-S and early-G_2 phase cells were unaffected by the drug. Survival decreased again when the cells moved into late-G_2 and G_1 phase. Cells exposed to higher concentrations of Yoshi 864 (75 and 100 μg/ml) showed a similar age-dependent response curve but with some degree of killing in late-S and early-G_2 phase.

The survival of T_1 cells as a function of increasing concentrations of Yoshi 864 incubated for 1 hour at selected stages of the cell cycle is shown in figure 4. All of the curves are "C" type and the greatest differences reside in the extent of the shoulder region which varies from a D_q of 10 μg/ml (1 hour) in early-S phase to a D_q of 50 μg/ml (1 hour) in late-S phase. The slopes of the various survival curves are largely similar.

Cell Cycle Progression

A. Asynchronous Cells

The effect of duration of treatment on progression through the mitotic cycle was analyzed for three concentrations, 10, 50, and 100 μg/ml. Cells were

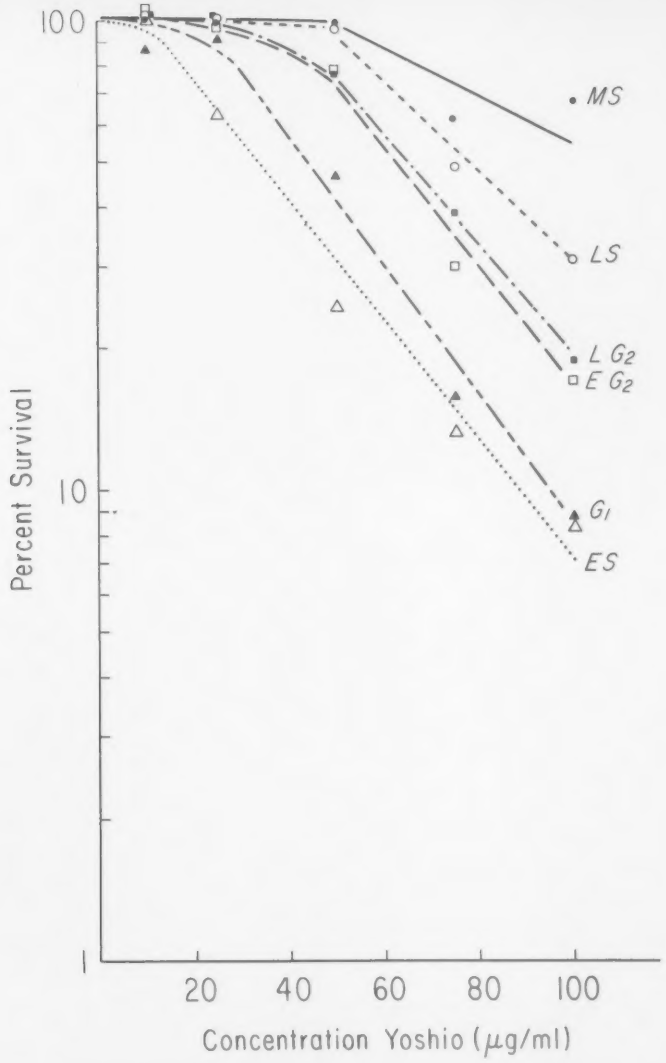

FIGURE 4.—Survival of synchronized T_1 cells treated with increasing concentrations of Yoshi 864 at selected stages of the cell cycle: ES = early-S; MS = mid-S; LS = late-S; EG_2 = early-G_2; LG_2 = late-G_2.

harvested both during drug exposure and after drug removal. Incubation of T_1 cells with 10 μg/ml did not significantly alter DNA distribution, even after exposure for 12 hours. Continuous treatment with 50 μg/ml of Yoshi 864 for 12 hours induced a slow steady increase of cells in S phase, while the G_1 compartment decreased at a similar rate (fig 5). The (G_2 + M) fraction did not increase until 12 hours after drug addition, with a concomitant rapid evacuation of the S-phase compartment and further decline of the G_1 pool. Peak values of (G_2 + M) accumulation occurred at 24 hours after drug addition, independent of the length of previous incubation with Yoshi 864. However, the amplitude and the span of the (G_2 + M) wave corresponded directly to these incubation periods. The MI at the time of (G_2 + M) peak levels never exceeded control values, indicating that the (G_2 + M) increment resulted only

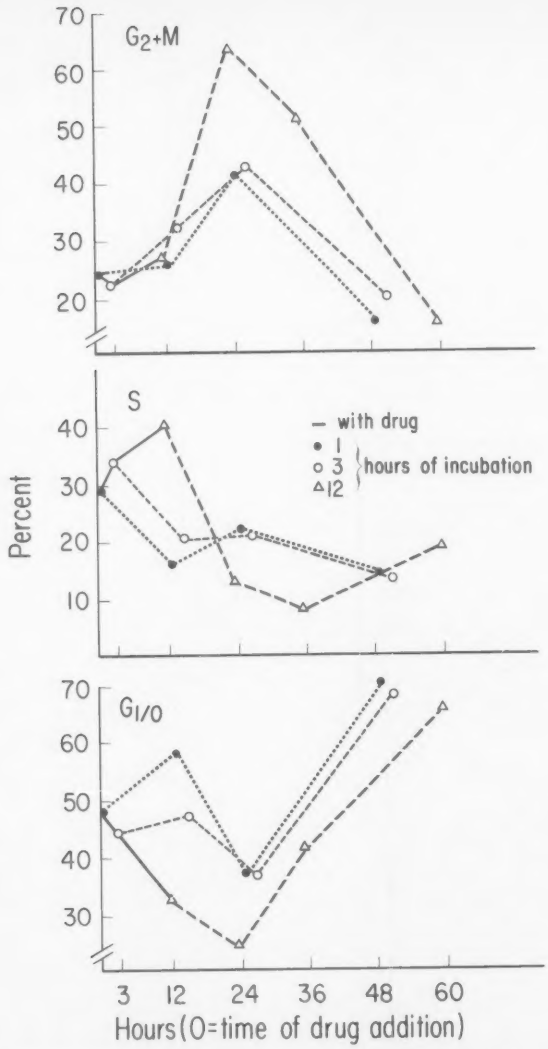

FIGURE 5.—Effect of treatment with 50 μg/ml of Yoshi 864 (1, 3, and 12 hrs of incubation) on the distribution of asynchronous T_1 cells in the various phases of the cell cycle. The solid line shows the compartment distribution in the presence of the drug. Postincubation followup is given by the dashed lines.

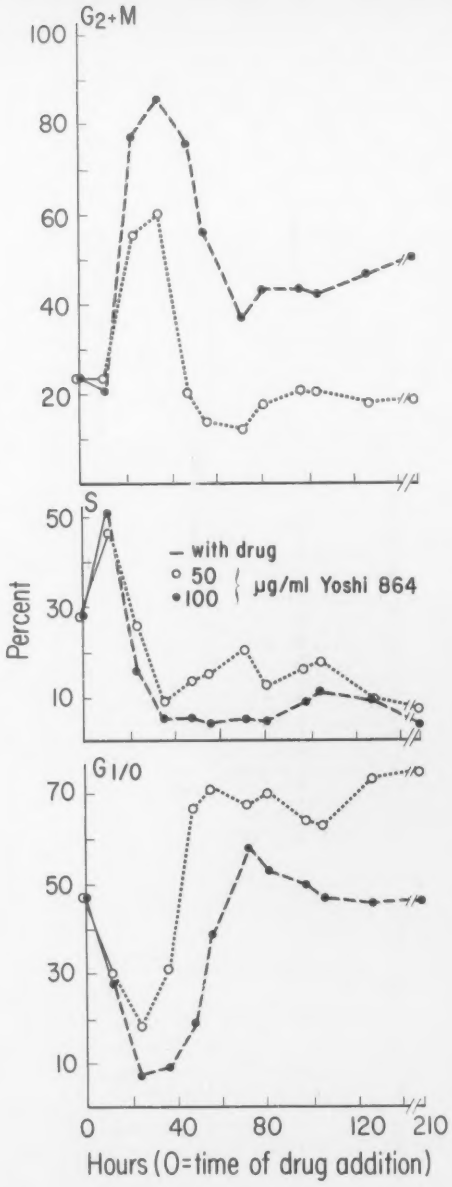

FIGURE 6.—Effect of 12 hrs of treatment with 50 and 100 μg/ml of Yoshi 864 on the distribution of asynchronous T_1 cells in the various phases of the cell cycle. The solid line shows the compartment distribution in the presence of the drug. Postincubation followup is given by the dashed lines.

from cells delayed in G_2 phase. G_2 accumulation lasted for several hours after which most cells entered G_1 phase as monitored by DNA histograms. A similar pattern of compartment shifts was observed for cells incubated with 100 μg/ml of Yoshi 864. However, magnitude and duration of G_2 accumulation increased for all exposure times. This effect can be readily visualized in figure 6 which shows a long-term followup analysis after a 12-hour treatment with 50 and 100 μg/ml of Yoshi 864. G_2 peak values of 60% (50 μg/ml) and 85% (100 μg/ml) were observed. The fraction of cells irreversibly arrested in the G_2 phase increased from 20% to 50% as a result

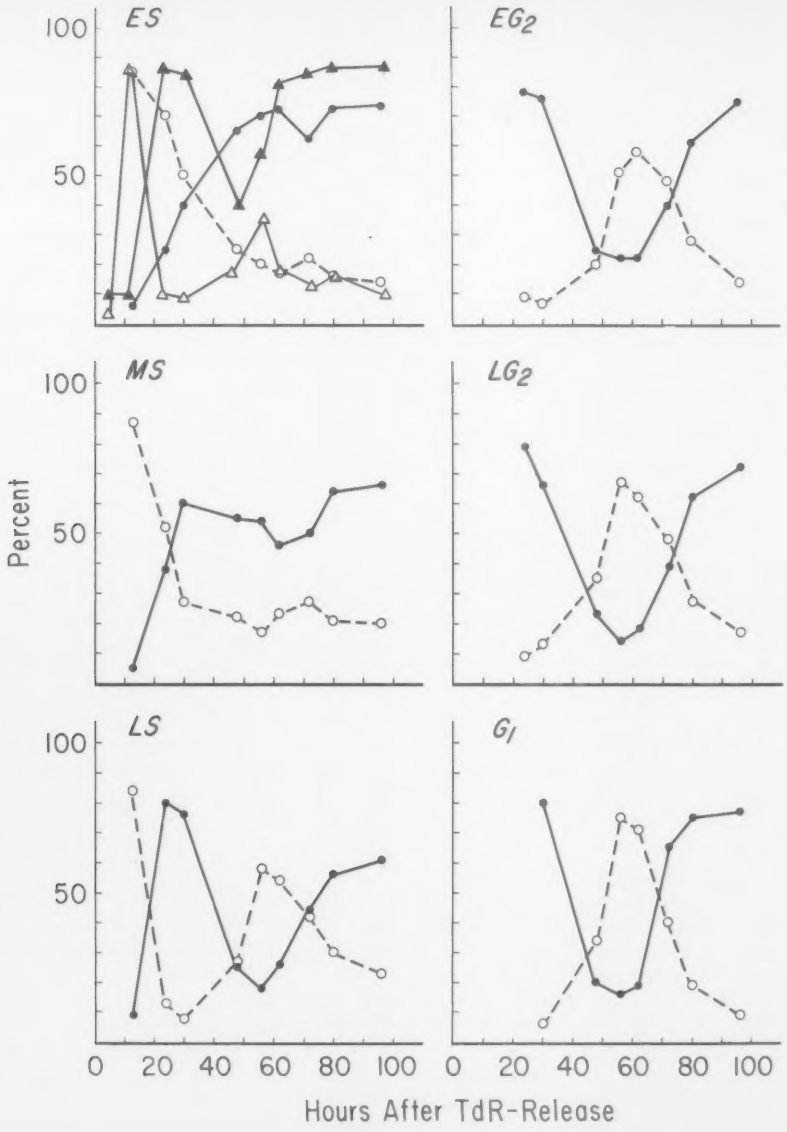

FIGURE 7.—Effect of 1 hr of treatment with 100 μg/ml of Yoshi 864 on the distribution of synchronized T_1 cells in G_1 (●——●) and G_2 (○------○) phases. The drug was added at 2 hrs (ES), 6 hrs (MS), 10 hrs (LS), 13 hrs (EG_2), 15 hrs (LG_2), and 24 hrs (G_1) after release of a 24-hr TdR block (3 mM). The compartment distribution in G_1 (▲——▲) and in G_2 (△——△) of control cultures is shown in the panel depicting cells treated in ES. To compare the sequence of compartment distributions in the subsequent panels, it is necessary to remember that control cultures show a G_2 peak of about 88% at 13 hrs after TdR release. By 24 hrs, the G_2 compartment reaches a minimum of 10%. This proportion is maintained until about 56 hrs, at which time control cells exhibit a second peak of 38% and drop to a minimum of 15% at 65–70 hrs. The G_1 compartment of control cells mirrors these changes. At about 24 hrs after TdR release, there is a peak of 85% cells in G_1 phase, which falls to about 40% at 48 hrs. A second wave of G_1 cells commences immediately after the second G_2 peak. Cells treated in ES and MS show a marked delay in the evacuation of the G_2 compartment; the minimum of 17%–20% is reached 32 hrs after control cells exited G_2. No second wave of G_2 cells is subsequently noted. In contrast, cells treated in LS and G_2 phase evacuate G_2 at the same rate as control cells. However, at the time when the second G_2 wave of control cells occurs, the treated cells exhibit significantly larger peaks (58%–75%). Populations treated in G_1 phase also showed a significantly larger wave of G_2 cells with respect to that of controls.

of the increase in concentration. There were no further compartment fluctuations of the progeny of cells perturbed by drug treatment. Prolongation of treatment to 24 or 48 hours did not change this kinetic response pattern.

B. Synchronized Cells

Analysis of cell cycle stage-dependent kinetic response was performed on synchronized cultures. At regular intervals after release of TdR block, cells were treated with 100 μg/ml of Yoshi 864 for 1 hour. Figure 7 depicts subsequent changes in G_1 (solid lines) and in G_2 (dotted lines) compartments for both control and treated cultures. Population distribution monitored at the time of arrival of control cells in early G_2 phase (13 hours after release of TdR block) was identical for cells that had been treated in early-, mid-, and late-S phase. This indicates that under these conditions, Yoshi 864 did not interfere with S-phase transit. However, the rate of egress out of G_2 depended on the stage of the cycle where treatment was applied. Cells treated in late-S phase exited the G_2 compartment with a rate similar to that of control cells, whereas the transit into the G_1 phase of cells treated in early- and mid-S phase was retarded. Thus, at the time that control cells and those treated in late-S phase had evacuated the G_2 compartment, G_2 fractions of 50% and 25% were still maintained for cells which had been treated in early- and mid-S phase. A G_2 minimum for the two latter populations was achieved at 56 hours (17%–20%), that is a delay of about 32 hours as compared to controls. There were only minor compartment fluctuations after division of cells treated in early- and mid-S phase. Compartment distribution of cells treated in early- and late-G_2 phase did not differ from control when monitored at the time of G_1 maximum (ie, 24 hours after TdR release). At the time of the second G_2 wave (38%) of control cells (56 hours), populations treated in late-S, G_2, and G_1 revealed G_2 peak values ranging between 58% and 75%. Progression into the subsequent G_1 compartment was delayed by 12 and 16 hours for populations treated in G_1 and G_2, respectively.

DISCUSSION

We examined the effects of the drug at the cellular level as an in vitro model of the lethal activity once the compound reaches the target cells in vivo. The survival curve obtained as a function of increasing concentrations was of the shoulder type typical of other alkylating agents (12) and ionizing radiation (13) on this cell line. The presence of a shoulder region suggests that the cells possess the capacity to absorb a certain degree of damage before expressing a significant killing effect. This capacity to absorb damage caused by Yoshi 864 is relatively small in comparison to that exhibited by T_1 cells treated with nitrosourea derivatives (Yoshi 864, D_{η} = 12 μg/ml; BCNU, 17 μg/ml; CCNU, 22 μg/ml; methyl-CCNU, 25 μg/ml) (13). However, the slope of the linear part of the curve, which reflects the sensitivity of the critical target molecule, is much shallower in the case of Yoshi 864 (48 μg/ml) than those obtained for the nitrosoureas: (BCNU, 17 μg/ml; CCNU, 11.5 μg/ml; methyl-CCNU, 19.5 μg/ml) indicating a threefold increase in resistance of the molecular target site. This independence between capacity to absorb damage and sensitivity of the target molecule is definitely demonstrated in stage-dependent response experiments. The survival curves obtained in these experiments indicate that cell cycle-dependent fluctuations in sensitivity result primarily from changes in the capacity to absorb damage (extent of the shoulder region) while target sensitivity (slope) remains practically unchanged. Thus, the cell cycle-dependent effect of Yoshi 864 is comparable to that obtained with ionizing radiation (12) and CCNU (13) on the same cell line, but differs from that observed for T_1 cells treated with BCNU and methyl-CCNU (13) where changes in both capacity to absorb damage and target sensitivity were noted. This information suggests that even though all of these agents may have a similar radiomimetic alkylating mechanism of action, the mode and probably the target molecule by which the lethal effect comes about differ in each.

In our present studies, Yoshi 864 induced a transient concentration and exposure time-dependent block of the cell cycle progression through S phase while the rate of progression from G_1 into S phase was unperturbed. This effect was more pronounced during continuous incubation for 12 hours and absent after treatment for 1 hour. After this effect, a largely reversible block in the G_2 phase developed. Both the extent and the duration of the G_2 block increased with concentration and exposure time for periods of up to 12 hours. The observation of a maximum G_2 block after a 12-hour incubation with no further increase in effectiveness after longer incubation times may be due to the biologic instability of the drug. The degree of recovery from the G_2 block is inversely related to drug concentration; thus, for a drug exposure of 12 hours, perturbation with 100 μg/ml resulted in an irreversibly arrested G_2 population twofold to threefold larger than that of cells treated with 50 μg/ml.

In experiments involving synchronized populations, only cells treated in G_1, early-, and mid-S

phase had a prolonged G_2 transit within the lifespan of the treated cells. When treatment was initiated in late-S or G_2 phase the rate of progression into mitosis was not affected, but when monitored for the subsequent life cycle, the amplitude and duration of the G_2 wave were considerably greater as compared to that of control cells. Thus, a G_2 delay is an obligatory event that will result independent of the position in the cell cycle where treatment with Yoshi 864 is applied. However, development of this effect necessitates a given period of time and, therefore, manifestation of this response may occur either within the same cell cycle of cells treated in G_1 and early- or mid-S phase or the delay is evidenced in the immediate progeny of cells treated in late S and G_2. This kinetic effect is short-lived, since after nearly complete recovery from the G_2 block no second G_2 wave is observed in the progeny of the perturbed cells.

An important question concerns the interrelation between kinetic response and cytotoxicity. In our studies with adriamycin, we suggested that the induction of a G_2 block could be utilized as a predictive test for cell lethality, since this effect was largely irreversible (15). For Yoshi 864, neither the extent of the G_2 block nor the fraction of cells irreversibly arrested in G_2 correlates with cell kill assessed by colony formation. Furthermore, the kinetic effect of cell progression delay in G_2 phase observed in the immediate progeny does not imply impaired reproductive capacity. Thus, kinetic distribution profiles after treatment with antitumor agents may not always be a reliable index of cellular reproductive death. However, it is noteworthy that the stages of the cell cycle most sensitive to the lethal effect of Yoshi 864 (G_1 and early S) were also those in which the subsequent G_2 block was manifested during the lifespan of the treated cells.

In conclusion, our data indicate that Yoshi 864, although a rather inefficient drug in terms of cell kill, may represent a useful synchronizing agent to be utilized in clinical protocols based on a kinetic rationale.

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Inhibition of Growth and Regression of a Transplantable Rat Chondrosarcoma by Three Retinoids [1]

P. W. Trown,* M. J. Buck, and R. Hansen [2]

SUMMARY

An aromatic analog of retinoic acid, *all-trans*-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid (Ro 10–1670), its ethyl ester (Ro 10–9359), and ethyl amide (Ro 11–1430) have been shown to inhibit the growth of a transplantable rat chondrosarcoma. The inhibitions observed occurred over a range of tolerated doses. At the higher tolerated dose levels, significant regressions of already established tumors were observed. All three compounds were active when administered ip for 2 or 4 weeks. The ethyl ester, Ro 10–9359, and the ethyl amide, Ro 11–1430, were also active when administered for 4 weeks as dietary admixes. In the latter experiments, both compounds were equally effective at tolerated doses, but Ro 11–1430 was less toxic than Ro 10–9359 at higher doses.

[Cancer Treat Rep 60:1647–1653, 1976]

Vitamin A (retinol = β-*all-trans*-retinol) and vitamin A acid (β-*all-trans*-retinoic acid) have been shown in many studies with experimental animals to influence the development of a variety of epithelial tumors; both prophylactic and therapeutic effects were observed (1,2). In man, retinoic acid caused regression of premalignant lesions such as actinic keratoses (3), leukoplakias of the oral cavity (4), and urinary papillomas (5) and was reported to exert a therapeutic effect on basal cell carcinomas (3). More recently, other retinoids including Ro 10–9359 and Ro 11–1430 (fig 1) have been shown to have better therapeutic indexes in animals (6,7) and some preliminary clinical trials with one of these for treatment of psoriasis have been reported (8).

Until recently, in all animal studies reported, retinoids were effective only in the prevention of carcinogenesis and had no effect against malignant, transplantable tumors. In 1975, however, Heilman and Swarm (9) reported that the 13-*cis* isomer of retinoic acid inhibited the growth of a transplantable chondrosarcoma in rats. We have now extended their observation and, in the study reported here, show that three synthetic aromatic retinoids (Ro 10–1670, Ro 10–9359, and Ro 11–1430; fig 1) not only inhibit growth of the chondrosarcoma, but also cause regression of established tumors.

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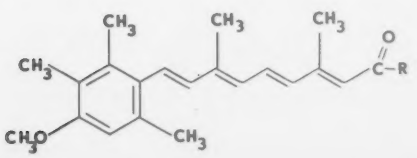

R	No.
OH	Ro 10—1670
OC_2H_5	Ro 10—9359
NHC_2H_5	Ro 11—1430

FIGURE 1.—Structures of retinoids.

MATERIALS AND METHODS

Tumor

The tumor used in these studies was obtained from Dr. R. L. Swarm as a serial transplant growing in a BUF/N rat. It was derived from a spontaneous chondroblastic osteogenic tumor of a female Sprague-Dawley rat (10) and has been maintained by sc implantation for > 10 years in BUF/N rats. Since acquisition in this laboratory it has been maintained in Fischer F344 rats. The tumor currently appears to be a well-differentiated chondrosarcoma.

Animals

Fischer F344 female rats (Charles River Breeding Labs, Wilmington, Mass) were used in this study. They were fed Purina Laboratory Chow (stated vitamin A content 24,000 IU/kg) ad libitum throughout the experiment.

Chemicals

All three retinoids used in this study were synthesized by and obtained from F. Hoffmann-La Roche and Co, Basle, Switzerland. The purity of each compound was > 98%.

Antitumor Testing

Tumors were removed from rats implanted 3–5 weeks previously and were minced aseptically. Several small pieces were implanted sc in the right inguinal region of rats (40–50 g) using a trocar.

The animals were kept for approximately 30–40 days at which time they were weighed, their tumors were measured by calipers, and groups of eight rats were randomly formed. Treatment was begun on the same day by either the ip or oral route daily, 5 times per week, or via dietary admixes. For ip and oral treatment the retinoids were suspended, with sonication, in 0.1% carboxymethylcellulose in water containing approximately 0.01% Triton X-100. A sufficient amount of each suspension was prepared for 1 week of treatment and stored in the dark at 4°C between uses. For administration in the diet, the retinoids were incorporated into gelatin beadlets containing sodium benzoate, ethoxyquin, and ascorbyl palmitate as anti-oxidants and stabilizers. The beadlets contained by weight 10.4% of Ro 11–1430 and 11.5% of Ro 10–9359 respectively. Placebo beadlets of identical composition but without retinoids were used for control animal diets. The beadlets were mixed in various amounts with Purina Laboratory Chow Meal and fed ad libitum to the treated animals. In the early experiments the consumption of food was measured for each rat. It was determined that the animals consumed approximately 10 g of food per day for each 100 g of body weight; subsequent daily intakes of food and drug were calculated on this basis.

The rats were weighed and their tumors measured at 2-week intervals. At the end of the experimental treatment period (2 or 4 weeks) the animals were killed and their tumors were excised and weighed. The inhibition of tumor growth was calculated as $\frac{C-T}{C} \times 100\%$ where C and T are the mean tumor weights for the control and treated animals

TABLE 1.—Inhibition of growth of a chondrosarcoma in Fischer rats by various retinoids—2-week studies

	Ro 10–1670 (ip)		Ro 10–9359 (ip)		Ro 11–1430 (ip)		Ro 11–1430 (oral)	
Dose* (mg/kg/day)	No. of survivors/ total No. of rats	% inhibition†	No. of survivors/ total No. of rats	% inhibition†	No. of survivors/ total No. of rats	% inhibition†	No. of survivors/ total No. of rats	% inhibition†
80	3/8	91.4	—	—	8/8	94.6	8/8	88.8
40	16/16	63.8	8/8	78.6	22/24	83.8	8/8	71.5
20	24/24	75.1	24/24	73.4	38/40	77.8	8/8	58.3
10	24/24	63.1	16/16	73.5	32/32	61.1	8/8	38.3
5	24/24	40.5	8/8	36.3	24/24	33.5	—	—
2.5	24/24	34.6	—	—	24/24	11.5	—	—

*Drugs were suspended in 0.1% carboxymethylcellulose in water containing a trace of Triton X-100 by sonication and administered daily, Monday-Friday, each week.

†Calculated using the formula $\frac{C-T}{C} \times 100\%$ where C = the mean tumor weight for control animals and T = the mean tumor weight for treated animals. Groups of 8 animals were used for each experiment and the % inhibitions for each experiment were averaged.

respectively. Inhibitions of ≥ 50% were considered to be evidence of antitumor activity.

The volumes of the tumors were calculated as the product of the maximum diameter in three perpendicular dimensions, and the mean volumes for each group were determined. The statistical significance of decreases in tumor volume, where observed, was determined using a two-sided t test.

RESULTS

2-Week Studies

All three retinoids, Ro 10–1670, Ro 10–9359, and Ro 11–1430, inhibited the growth of the chondrosarcoma (table 1). Inhibition (> 50%) was observed for all three compounds administered ip at three dose levels (40, 20, and 10 mg/kg/day). Ro 10–1670 and Ro 11–1430 were also active at 80 mg/kg/day, but Ro 10–1670 appeared to be toxic, with only three of the eight animals surviving. Of the three compounds, only Ro 11–1430 was tested orally by intubation; it was active when administered for 2 weeks at 80, 40, and 20 mg/kg/day, but the degree of inhibition observed at each dose was lower than for the same dose administered ip.

4-Week Studies

Ip Administration

Tumor inhibition.—The effects of ip administration of the three retinoids on chondrosarcoma-bearing rats are summarized in table 2. In general, greater inhibitions of tumor growth were observed for given doses of all three drugs than for the same doses administered ip for only 2 weeks. In addition, activity was observed at doses of 5 and 2.5 mg/kg/day for Ro 11–1430 and 5 mg/kg/day for Ro 10–1670; at these doses neither com-

TABLE 2.—Inhibition of growth of a transplantable chondrosarcoma by various retinoids administered ip—4-week studies

Compound	Approximate dose (mg/kg/day)*	Experiment No.	% tumor inhibition*	No. of survivors/ total No. of rats	Mean weight changes (g)		Mean tumor volume			Significance of regression on Day 29
					Body and tumor†	Body†	Day 1†	Day 15†	Day 29†	
Ro 11–1430	20	2	94.3	5/8	+26.6 S=6.3	+26.0 S=6.3	1143 S=418	903 S=401	536 S=433	$P = 0.05$
	20	7	91.2	8/8	+10.1 S=4.5	+8.5 S=4.7	5021 S=2470	2668 S=1500	1827 S=728	$P < 0.01$
	10	7	87.4	8/8	+17.9 S=5.7	+15.6 S=5.8	3069 S=1149	4022 S=2979	3250 S=2029	—
	5	7	65.2	8/8	+21.6 S=5.1	+15.4 S=6.4	3425 S=1167	7807 S=4333	8681 S=3597	—
	2.5	7	51.0	8/8	+24.0 S=7.7	+15.2 S=6.9	2531 S=887	7889 S=2311	12219 S=3501	—
Ro 10–1670	40	2	94.3	5/8	−29.9 S=17.9	−30.5 S=17.9	1218 S=503	1309 S=429	598 S=302	$P < 0.02$
	20	7	94.1	7/8	−9.6 S=17.0	−10.6 S=16.8	3685 S=1429	1245 S=590	1117 S=402	$P < 0.002$
	10	7	86.1	8/8	+14.3 S=10.9	+11.8 S=10.5	3729 S=1334	3891 S=1265	2812 S=1190	$P > 0.1$
	5	7	65.6	8/8	+25.8 S=6.1	+19.6 S=6.6	3259 S=1077	5688 S=2265	8686 S=4008	—
	2.5	7	48.2	8/8	+24.6 S=7.8	+15.3 S=8.6	3876 S=1984	8840 S=4589	11903 S=5279	—
Ro 10–9359	10	2	94.0	6/8	+16.6 S=17.5	+16.0 S=17.6	1150 S=303	840 S=390	415 S=139	$P < 0.002$
Controls	—	2	—	8/8	+40.4 S=7.7	+29.7 S=5.2	1161 S=512	5610 S=2608	11272 S=6323	—
	—	7	—	8/8	+31.3 S=5.2	+13.3 S=3.8	3270 S=1455	12376 S=3679	24950 S=7339	—

*See table 1.
†S = standard deviation.

pound was active following 2 weeks of treatment. Ro 11–1430 appeared to be tolerated at the highest dose tested (20 mg/kg) as judged by the fact that the surviving animals gained weight. In contrast, at this same dose Ro 10–1670 appeared to be less well tolerated since surviving animals showed a significant loss of body weight. At the 10- and 5-mg/kg/day levels where tumor inhibition but not regression was noted, the compound appeared to be tolerated. At the one dose tested (10 mg/kg/day), Ro 10–9359 did not appear to be toxic since the surviving animals gained weight.

Tumor regression.—In all experiments reported here, tumors were allowed to grow for 20–40 days after implantation before treatment began. Thus, it was possible to observe not only inhibition of further tumor growth but also regression of the established tumors. Significant regressions ($P < 0.2$ or better) were observed at 20 mg/kg/day for Ro 11–1430, at 40 and 20 mg/kg/day for Ro 10–1670, and at 10 mg/kg/day for Ro 10–9359 (table 2).

Administration in the Diet

When Ro 10–9359 and Ro 11–1430 were adminis-

TABLE 3.—Effects of Ro 11–1430 administered in the diet to Fischer rats bearing a transplantable chondrosarcoma—4-week studies

Approximate dose (mg/kg/day)*	Experiment No.	% tumor inhibition†	No. of survivors/ total No. of rats	Mean weight changes (g)		Mean tumor volume			Significance of regression on Day 29
				Body and tumor‡	Body‡	Day 1‡	Day 15‡	Day 29‡	
80	12	98.1	8/8	−24.6 S=3.4	−25.1 S=3.4	3317 S=1196	1907 S=994	439 S=388	$P < 0.001$
40	12	97.4	8/8	−17.8 S=9.8	−18.5 S=9.8	5804 S=1456	2768 S=641	653 S=183	$P < 0.001$
40	13	96.7	8/8	−22.9 S=4.5	−23.4 S=4.5	2105 S=996	554 S=400	264 S=287	$P < 0.001$
20	13	96.3	8/8	−6.6 S=5.3	−7.1 S=5.3	2023 S=1219	472 S=336	310 S=169	$P < 0.01$
20	15	94.9	8/8	0.0 S=4.3	−0.9 S=4.3	5329 S=3528	1390 S=996	704 S=548	$P < 0.01$
10	15	90.1	8/8	+1.5 S=5.6	−0.3 S=5.6	5868 S=2870	3301 S=1060	1401 S=471	$P < 0.01$
10	22	94.2	8/8	+12.5 S=2.9	+11.9 S=2.9	2298 S=992	853 S=615	555 S=220	$P < 0.002$
5	15	74.5	8/8	+5.7 S=11.2	+1.1 S=11.4	6702 S=4019	6566 S=4050	4549 S=2438	$P > 0.1$
5	22	82.2	8/8	+16.8 S=4.2	+15.1 S=4.4	2602 S=1146	2895 S=1635	2020 S=1792	$P > 0.1$
2.5	15	54.5	8/8	+11.2 S=5.2	+3.0 S=6.9	5909 S=4572	7921 S=4638	8577 S=4770	—
2.5	22	62.8	8/8	+19.0 S=4.9	+15.4 S=5.0	2172 S=909	4093 S=1732	4599 S=1694	—
1.25	22	35.5	8/8	+20.0 S=6.5	+13.7 S=8.4	2215 S=913	5622 S=2215	9563 S=5178	—
Control	12	—	8/8	+31.0 S=14.6	+3.2 S=11.5	4424 S=2409	30409 S=20722	36765 S=32364	—
	13	—	8/8	+27.4 S=3.5	+13.7 S=4.9	2272 S=899	7522 S=1870	17588 S=6524	—
	15	—	8/8	+11.8 S=5.9	−6.2 S=9.3	6609 S=2954	11755 S=4893	22464 S=8922	—
	22	—	8/8	+21.3 S=6.3	−10.2 S=8.9	2222 S=841	7094 S=2671	15650 S=4978	—

*Drugs were administered in gelatin beadlets as dietary admixes. Doses are calculated assuming that the animals each receive 10 g of food/day for each 100 g of body weight (*see* Materials and Methods).

†*See* table 1.

‡S = standard deviation.

TABLE 4.—Effects of Ro 10-9359 administered in the diet to Fischer rats bearing a transplantable chondrosarcoma—4-week studies

Approximate dose (mg/kg/day)*	Experiment No.	% tumor inhibition*	No. of survivors/ total No. of rats	Mean weight changes (g)		Mean tumor volume			Significance of regression on Day 29
				Body and tumor†	Body†	Day 1†	Day 15†	Day 29†	
80			0/8						
40			0/8						
20			0/8						
10	15	92.5	8/8	−15.3 S=10.5	−16.6 S=10.5	7367 S=4538	3941 S=3128	933 S=534	$P < 0.01$
10	22	95.1	8/8	−21.0 S=9.9	−21.5 S=10.0	2106 S=1313	497 S=312	310 S=149	$P < 0.01$
5	15	80.1	8/8	+1.4 S=10.3	−2.2 S=13.2	6401 S=3814	5594 S=3198	3012 S=1421	$P < 0.05$
5	22	86.3	8/8	+20.8 S=6.3	+19.5 S=6.4	2793 S=808	2374 S=1312	1544 S=949	$P < 0.02$
2.5	15	63.2	8/8	+11.8 S=5.5	+5.1 S=7.8	4014 S=1768	7610 S=3563	6155 S=3226	—
2.5	22	69.0	8/8	+21.3 S=3.4	+18.3 S=4.1	2492 S=1398	3858 S=2832	3681 S=2845	—
1.25	22	47.4	8/8	+20.5 S=4.5	+15.3 S=4.3	2328 S=1075	4841 S=1438	9143 S=3674	—
Control	15	—	8/8	+11.8 S=5.9	−6.2 S=9.3	6609 S=2954	11721 S=4900	22464 S=8992	—
Control	22	—	8/8	+21.3 S=6.3	+11.5 S=8.9	2222 S=841	7094 S=2671	15650 S=4978	—

*See table 3.
†S = standard deviation.

tered as dietary admixes, significant inhibitions of tumor growth and regressions of established tumors were observed (tables 3 and 4).

Tumor inhibition.—Both Ro 11-1430 and Ro 10-9359 caused inhibition of tumor growth of ≥ 50% at doses of 10, 5, and 2.5 mg/kg/day (tables 3 and 4). However, at doses of ≥ 20 mg/kg/day no animals survived treatment with Ro 10-9359, whereas all animals survived treatment with Ro 11-1430 up to the highest dose tested (80 mg/kg/day). Nevertheless, treatment with Ro 11-1430 at the higher doses did cause considerable loss of body weight indicating possible toxicity. At 20 mg/kg/day there was minor body weight loss and at 10 mg/kg/day there was a slight loss in one experiment and a gain in the other. At ≤ 5 mg/kg/day animals gained weight. The significance of the changes in body weight at ≤ 20 mg/kg/day is difficult to assess because in two of the four control groups the animals lost body weight when correction for the weight of the tumor was made. In the other two control groups the animals gained weight. Ro 10-9359 was more toxic than Ro 11-1430 in these experiments; no animals survived the treatment period at 80, 40, and 20 mg/kg/day. At 10 mg/kg/day there was severe weight loss but at 5, 2.5, and 1.25 mg/kg/day the compound appeared to be well tolerated, judging by the changes in body weight.

Tumor regression.—Significant regressions ($P < 0.01$ or better) of established tumors occurred after treatment with Ro 11-1430 at 80, 40, 20, and 10 mg/kg/day and with Ro 10-9359 at 10 and 5 mg/kg/day (tables 3 and 4). For each compound there was at least one dose level at which regression occurred without appreciable effects on changes in body weight (20 and 10 mg/kg/day for Ro 11-1430 and 5 mg/kg/day for Ro 10-9359).

DISCUSSION

The original observation by Heilman and Swarm of the inhibition of growth of a transplantable rat chondrosarcoma by 13-*cis*-retinoic acid (9) has now been extended to three aromatic retinoids, Ro 10-1670, Ro 10-9359, and Ro 11-1430 (fig 1). In addition, these compounds have been shown

to produce significant regressions of established tumors growing logarithmically at the time treatment was begun.

The three subject retinoids are closely related structurally: Ro 10–9359 is the ethyl ester and Ro 11–1430 is the ethyl amide of the *all-trans*-retinoic acid analog Ro 10–1670 (fig 1). Their activities against this chondrosarcoma are also very similar, particularly in the 2-week treatment protocol experiments (table 1). Extension of the treatment protocol to 4 weeks revealed differences in toxicity between the acid Ro 10–1670 and its ethyl amide Ro 11–1430. Though tumor inhibitions and regressions were very similar at the doses tested ip, Ro 10–1670 appeared to be more toxic than its ethyl amide (Ro 11–1430). Insufficient experiments were carried out with the ethyl ester Ro 10–9359 administered ip to make comparisons with the other two compounds.

Only the ethyl ester (Ro 10–9359) and its ethyl amide (Ro 11–1430) were tested as dietary admixes and in these experiments the ethyl amide was clearly superior (tables 3 and 4). Both compounds caused similar inhibitions of tumor growth at doses of 2.5, 5, and 10 mg/kg/day and tumor regressions at 10 mg/kg/day. However, Ro 10–9359 caused appreciable weight loss at 10 mg/kg/day and death of all the animals in groups fed 20, 40, and 80 mg/kg/day. Ro 11–1430 caused severe weight loss only at doses of 40 and 80 mg/kg/day and in contrast to Ro 10–9359, no deaths were observed in any of the treatment groups, even at doses of 80 mg/kg/day.

The toxic effects observed at high doses of any of the three compounds are similar to those observed at high doses of vitamin A (eg, weight loss, exudate around the eyes, bone fractures). As with vitamin A, these symptoms disappear rapidly after treatment is discontinued.

The mechanism by which these retinoids inhibit tumor growth is unknown. However, Shapiro et al (11) recently reported that explants of chondrosarcoma from rats that had been treated with retinoids in this present study showed a decreased ability to fix $^{35}SO_4$ into glycosaminoglycan relative to explants from untreated rats. In contrast, the incorporation of $^{35}SO_4$ into glycosaminoglycan by costal cartilage from the same retinoid-treated tumor-bearing animals was similar to that of costal cartilage from the untreated control rats. The differing sensitivity of normal and neoplastic cartilage to retinoid therapy may result from differing bioavailability of the retinoids to the two types of cartilage. A specific retinoic acid-binding protein (12,13) which was detected in this chondrosarcoma[3] but was not measurable in a normal cartilage[4] may result in a higher concentration of retinoids in the neoplastic cartilage. Alternatively, the retinoids may be acting at the level of cellular differentiation, as in the case of epithelial lesions.

The specific inhibition of glycosaminoglycan synthesis in neoplastic cartilage results in loss of matrix by the tissue and may thus render it susceptible to immunologic rejection. Recently, Langer and Gross (14) showed that intact cartilage explants were not immunogenic whereas free chondrocytes or shavings of cartilage were. Heyner (15) has also concluded that the matrix of cartilage prevents rejection of chondrocytes in allografts of intact cartilage. It would therefore be of great interest to study the effects of these retinoids on a syngeneic chondrosarcoma where immunologic rejection should not occur. Such studies would also be useful in assessing the potential usefulness of these compounds for the treatment of chondrosarcoma in man.

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Phase II Trial of Cytembena in Patients With Advanced Ovarian and Breast Cancer[1,2,3]

H. C. Falkson* and G. Falkson[4]

SUMMARY

Thirty women with histologically proven advanced ovarian or breast cancer were treated with cytembena. Of nine patients with ovarian cancer and 21 with breast cancer none had worthwhile objective remissions. Cytembena was given at a dose of 250 mg/m²/day for a course of 5 days repeated at weekly intervals. Improvement, particularly relief of pain from skeletal metastases, was observed in 16 of the patients; an additional seven patients had stable disease while treated with cytembena. Cytembena has no hemopoietic toxicity, but nausea and vomiting and an "autonomic storm" phenomenon are dose-limiting factors. Further studies incorporating this drug in combination regimens seem warranted.

[Cancer Treat Rep 60:1655–1658, 1976]

Cytembena, a sodium β-4-methoxybenzoyl β-*cis* bromoacrylate, was originally developed by Czechoslovakian investigators. It is a hydrolytic product of antineoplastically active crotonolactones, with a chemical structure unrelated to any other antineoplastic agents.[5] It is structurally similar to norepinephrine, the adrenergic neurotransmitter. Its mechanism of action is thought to be inhibition of purine biosynthesis, inhibition of tetrahydrofolate formylase, and inhibition of incorporation of amino acids including glutamic acid, glycine, and methionine. It has been shown to be active against various experimental animal tumors and Czechoslovakian investigators reported encouraging results with this agent in the treatment of gynecologic malignancies, particularly ovarian cancer (1). According to these reports cytembena was associated with no hematologic, hepatic, or renal toxicity. The agent only became available for clinical trial in the US and South Africa in 1974. Investigators from the Mayo Clinic, Rochester, Minn, recently reported on phase I and II trials with cytembena (2,3). In 1974 we started a clinical trial with cytembena and the purpose of this paper is to report our results with this agent in patients with advanced ovarian and breast cancer.

MATERIALS AND METHODS

Thirty women with an average age of 52 years (range, 34–68 years) received one or more courses of cytembena. Nine patients with ovarian cancer and 21 with breast cancer were entered in this study. All patients had histologically confirmed, advanced metastatic cancer and all had undergone menopause, either natural or induced. Entry in the study required all patients to have measurable disease progression, a normal blood urea, creatinine of < 1.5 mg/100 ml, and bilirubin of < 2.0 mg/100 ml. Patients with leukopenia and/or thrombocytopenia were also eligible for the study.

All nine patients with ovarian carcinoma had extensive abdominal metastases; in addition, two had liver metastases and one had inguinal gland involvement. Of the 21 patients with breast cancer 19 had diffuse, painful skeletal metastases; nine of these patients had visceral and/or subcutaneous and skin involvement in addition to the skeletal metas-

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[2]Supported by a grant from the National Cancer Association of South Africa.

[3]Cytembena: NSC-104801; CAS reg. No. 16170-75-5; acrylic acid, 3-*p*-anisoyl-3-bromo-, sodium salt. Supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute (NCI).

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[5]Slavik M. Cytembena (NSC-104801): an updated review. Prepared by the NCI, Bethesda, Md, Mar 1972.

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tases. Two patients had only skin and lymph gland metastases. All but one of the patients had previously received extensive prior chemotherapy with multiple-drug regimens including cyclophosphamide, methotrexate, 5-fluorouracil, adriamycin, and dibromodulcitol in patients with breast cancer. A 64-year-old woman with abdominal and liver metastases from an adenocarcinoma of the ovary received cytembena as her first treatment.

Cytembena is supplied as a white, water-soluble powder in 200-mg vials. It can be administered iv, im, or ip. The majority of patients received the drug iv. Because of unpleasant subjective reactions encountered in patients who received the agent as a direct injection, the agent was usually given in normal saline as a slow iv infusion over a period of half an hour. In six of the patients with inaccessible veins the im route was used in addition to iv infusions. In one 47-year-old woman with rapidly filling massive ascites ip administration was also used but it was not repeated because of exquisite abdominal pain experienced immediately after cytembena administration.

Cytembena was given at a dose of 250 mg/m²/day for 5 days. The course was repeated weekly as long as the patient tolerated it subjectively. Maintenance therapy was given at 3-week intervals. The total dose administered to patients with ovarian cancer varied from 36,300 mg given over a period of 62 days to 2400 mg given over 5 days. The median total dose administered to ovarian cancer patients was 6200 mg over a period of 30 days. The total dose administered to patients with breast cancer varied from 13,600 mg over a period of 120 days to 1000 mg over 5 days. The median total dose administered to breast cancer patients was 3600 mg over a period of 120 days.

At the start of treatment the performance status (PS) of each patient was recorded as follows: 0 = normal activities; 1 = mild impairment of performance ability; 2 = moderate impairment of performance but < 50% of the time spent in bed; 3 = severe impairment of performance and > 50% of the time spent in bed; and 4 = very ill, bedridden patient. Of the nine patients with ovarian cancer six had a PS of 3 and three had a PS of 2. Of the 21 patients with breast cancer one had a PS of 4 (bedridden because of a pathologically fractured femur), nine had a PS of 3, five had a PS of 2, five had a PS of 1, and only one had a normal PS (0).

The following laboratory tests were done before the start of treatment with cytembena, on the last day of a course of treatment, and at 3-week intervals thereafter: cbc count including reticulocytes, alkaline phosphatase, SGOT, serum electrophoresis, calcium and phosphorus, bilirubin, creatinine, urea, uric acid, blood sugar, and urinalysis.

RESULTS

In evaluating the therapeutic response the following criteria were used. For CR the total regression of measured lesions and the disappearance of all other objective evidence of disease were required; for PR an average decrease of all measurable disease of ≥ 50% was required. Any response less than that considered to be CR or PR was designated improvement; if the condition remained stable while being treated with cytembena it was recorded as no change. Progressive disease was considered an indication that the treatment should be changed immediately. No CRs or PRs were observed.

Ovarian Cancer

In four of the nine patients with ovarian cancer marked improvement of pain occurred during the period of cytembena administration. In one of these patients (a 47-year-old woman with massive ascites requiring repeated paracentesis) one administration of ip cytembena in addition to systemic cytembena controlled the ascites for 3 weeks. In another patient (a 34-year-old woman) marked improvement of pain was observed for 4 months during cytembena therapy. She was given a total dose of 7600 mg of cytembena iv over a period of 80 days. Three other women had stable disease; in one of these patients stable disease lasted up to 62 days. Two women received only one and two courses respectively; in both patients cytembena was discontinued because of rapidly progressing disease. Only two of the nine patients responded to subsequent chemotherapy given after cytembena. A 58-year-old woman who showed progression of disease with the combination treatment of 5-fluorouracil, methotrexate, and cyclophosphamide had stabilization of disease on cytembena for 62 days; she had a PR due to other cytostatics administered after the cytembena treatment and survived for 1 year and 2 months from the start of the cytembena trial. A 64-year-old woman who had no previous chemotherapy showed progression of disease with cytembena and subsequently responded to other cytostatics; she is still alive 1 year after the start of cytembena therapy.

Breast Cancer

In 12 of the 21 breast cancer patients marked relief of bone pain occurred. The pain relief was sometimes so dramatic that patients did not need any analgesics. However, this relief of pain usually

lasted only while cytembena was being given, and because no evidence of objective improvement occurred, treatment was usually changed to other cytostatics. In a 50-year-old woman bone pain as well as large painful glands subsided, but in none of the other patients could improvement of soft tissue lesions be ascribed to cytembena therapy.

Four patients had no change of disease and experienced no relief of bone pain. Five women had progression of disease after one to three courses of cytembena; all five had skin as the dominant metastatic site.

TOXICITY

No hematologic toxicity was observed. Four of the patients received cytembena despite leukopenia and thrombocytopenia. Wbc and platelet counts usually returned to normal during cytembena treatment. In a 52-year-old woman who received cytembena despite a wbc count of 1800 cells/mm^3 and a platelet count of 86,000 platelets/mm^3, the wbc count recovered to 4600 cells/mm^3 and the platelet count recovered to 155,000 platelets/mm^3 after 33 days of cytembena treatment.

At the dose range used in this study (250 mg/m^2), the major toxic effects were nausea and vomiting. Two thirds of the patients experienced moderate to severe nausea with or without vomiting. In most instances the administration of im chlorpromazine at the time of cytembena administration, as well as giving the drug by slow iv infusion, counteracted these side effects.

Arm pain, beginning at the site of injection and advancing to the shoulder and chest, was reported by three patients. Another three patients complained of facial discomfort and burning and soreness of the gums within 15 minutes after the injection. Two patients had nasal mucosal burning and one of these had severe attacks of sneezing within half an hour of every cytembena injection. Antihistaminic agents were of very little use in controlling this side effect.

Two of the patients fainted within half an hour of cytembena injection and had to be hospitalized for subsequent treatments. Another woman became very agitated 10 minutes after administration of cytembena and stated a fear of impending death. Intensity of these subjective observations was much more severe when a single dose > 400 mg was administered. No changes in liver functions were observed, and not even transient abnormalities of urea, uric acid, or creatinine occurred. In one third of the patients transient glycosuria was recorded during the week of cytembena therapy, but blood sugar values were within normal range.

DISCUSSION

Czechoslovakian investigators reported encouraging results with cytembena in patients with gynecologic malignancies. They reported a 30% 5-year survival in women with stage III and IV ovarian cancer (1). However, in analyzing their results it appears that they usually gave cytembena in conjunction with an alkylating agent. In a phase II study by the investigators at the Mayo Clinic (3) cytembena was compared to VP-16-213 in patients with advanced breast cancer. They observed no objective remissions in any of the patients in their study. Of 15 women treated with cytembena, five had stability of disease for a mean duration of 10 weeks. Our own results are comparable to those of the Mayo Clinic: we did not record any objective remissions. However, marked pain relief occurred in a large number of patients treated with cytembena and stable disease was observed in 23% of the patients. The possibility that the pain relief encountered with cytembena may be due to the presence of bromine in this drug must not be overlooked. The mean survival for the patients in our study after cytembena therapy was 4.8 months for patients with ovarian cancer and 7.2 months for those with breast cancer. Cytembena was a useful agent in some patients with breast cancer with diffuse and painful skeletal metastases. Of practical importance was the fact that cytembena could be given to patients who had a poor bone marrow reserve; when leukocytes and thrombocytes had recovered to normal range these patients could again be treated with other cytostatics. From our results in ovarian cancer cytembena does not seem to have a worthwhile role in the treatment of this disease.

Cytembena is a drug of unique chemical structure and although it does not cause hemopoietic depression it has a wide variety of side effects, suggesting an increased activity of the autonomic nervous system. Frytak et al (2) observed elevated catecholamines in patients undergoing treatment with cytembena, and they have suggested that this elevation may be responsible for the "autonomic storm" phenomenon seen in many of the patients during treatment with this agent. In view of the disappointing therapeutic results observed with cytembena it is unlikely that this agent will be used further in extensive clinical trials. Studies directed toward the drug's interaction with the autonomic system may prove beneficial.

The significant numbers of patients who experienced stable disease, both in our study and also in that of the Mayo Clinic, indicate some antitumor effect for cytembena in human neoplastic disease. Since cytembena has no hematologic toxicity the

possibility of incorporating this drug in combination treatment is very practical; its usefulness could therefore be explored in combinations with other cytostatic agents in the treatment of metastatic disease.

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Phase II Study of Methyl-CCNU in the Treatment of Advanced Pancreatic Carcinoma [1,2]

Brief Reports and Preliminary Communications

Charles G. Moertel,* Harold O. Douglass, James Hanley, and Paul P. Carbone [3]

Carcinoma of the pancreas is a challenge of singular importance to medical oncologists in this country. Its increase in reported incidence over the last two decades has been exceeded only by lung cancer. Pancreatic cancer now ranks fourth in cancer mortality and is predicted to be responsible for > 21,000 deaths during 1976 (1). Early diagnosis and surgical treatment of this disease have been essentially fruitless and almost all patients become candidates for medical treatment of unresectable carcinoma.

In spite of this great need, research in the chemotherapy of pancreatic cancer has been badly neglected. In 1974, Comis and Carter (2) could find only six drugs for which a total treatment experience exceeding 15 patients each had been recorded: 5-fluorouracil (5-FU), mitomycin C, benzcarbamine, BCNU, CCNU, and streptozotocin. A clear and evident need exists for exploration of the therapeutic efficacy of both new and old chemotherapeutic agents in pancreatic cancer.

The nitrosoureas have shown definite, albeit limited, activity in gastrointestinal adenocarcinoma. In the overall pancreatic cancer experience with BCNU and CCNU, Comis and Carter (2) found three (5%) objective regressions among a total of 56 treated patients. Although this is hardly an enviable record, uncontrolled comparison at the Mayo Clinic (Rochester, Minn) (3) has suggested that for another gastrointestinal adenocarcinoma, large bowel cancer, methyl-CCNU may be the most favorable nitrosourea. The purpose of this ECOG study was therefore to evaluate the therapeutic potential of methyl-CCNU in patients with advanced and metastatic adenocarcinoma of the pancreas.

MATERIALS AND METHODS

All patients selected for study had histologically confirmed unresectable adenocarcinoma with the primary lesion established to be in the pancreas either by surgical exploration or by convincing clinical evidence. All patients were required to have clearly measurable areas of known malignant disease to serve as objective indicators of response to therapy. Malignant hepatomegaly was accepted as an indicator only if the liver extended a minimum of 5 cm below the costal margins on quiet respiration. Such vagaries as extrarectal shelves, widened duodenal loops, abdominal girth, pancreatic scans, and clips at the margins of tumors were not accepted. Therapy was deferred for ≥ 2 weeks after any abdominal surgery and for ≥ 3 weeks if a resection or bypass procedure had been performed. Therapy was also deferred for ≥ 1 month after any previous chemotherapy or radiation therapy. Characteristics of the patients and their tumors are shown in table 1.

Contraindications to selection included: previous chemotherapy with a nitrosourea, severe malnutrition, severe nausea or frequent vomiting, leukopenia (< 4500 cells/mm^3) or thrombocytopenia (< 150,000 platelets/mm^3), renal disease with azotemia (creatinine > 1.5 mg/100 ml), or significant impairment of liver function, ie, any elevation of the direct-reacting serum bilirubin. In an attempt to avoid selection of any specific group of patients for

[1]Received Sept 16, 1976; accepted Oct 20, 1976.

[2]Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

[3]This study was performed by the Eastern Cooperative Oncology Group (ECOG) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. The following institutions participated in the study: Mayo Clinic, Rochester, Minn (*C. G. Moertel*, CA-13650); Roswell Park Memorial Institute, Buffalo, NY (*H. O. Douglass*, CA-12296); Statistical Center, State University of New York, Buffalo (*J. Hanley*, CA-12721); ECOG, Silver Spring, Md (*P. P. Carbone, Chairman*, CM-33745).

Other participating institutions include: Albany Medical College (CA-06594); Albert Einstein College of Medicine (CA-14958); University of Alberta (CA-13314); American Oncologic Hospital (CA-18281); Baystate Medical Center; Boston University Medical Center (CA-11109); Case Western Reserve University (CA-14548); Chicago Medical School (CA-14144); Hahnemann Medical College (CA-13611); University of Iowa; Jackson Memorial Hospital (CA-02822); Baptist Memorial Hospital; Jefferson Medical College (CA-14215); University of Manitoba; Medical College of Virginia (CA-10572); Mt. Sinai Medical Center, NY; Northwestern Memorial Hospital (CA-17145); University of Ottawa and Ottawa Cancer Foundation; University of Pennsylvania (CA-15488); University of Rochester Cancer Center (CA-11083); Rush-Presbyterian St. Luke's Medical Center (CA-10948); University of Pretoria, H.F. Verwoerd Hospital.

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methyl-CCNU treatment, entry into this study was randomized with a second study evaluating combinations of streptozotocin with either 5-FU or cyclophosphamide. This latter study will be reported in a second publication.

Methyl-CCNU was administered orally in gelatin capsules after a 2-hour fast. A single dose of 200 mg/m^2 was employed. If the patient showed an objective response or remained objectively stable without significant clinical deterioration, therapy was repeated at 7-week intervals. Dosage was appropriately reduced if severe hematologic depression had been experienced with the preceding course. Leukocyte and platelet counts were recommended twice weekly after therapy. Results of treatment were evaluated at 7 weeks after the onset of therapy and at 7-week intervals thereafter. An objective response was declared if the most clearly measurable area of known malignant disease had shown a decrease of $\geq 50\%$ of the product of longest perpendicular diameters. If malignant hepatomegaly was the indicator lesion, it was required that the sum of measurements below the xiphoid and each costal margin at the midclavicular lines decrease by $\geq 30\%$. There could be no increase in other areas of known malignant disease and no new areas of malignant disease could appear.

TABLE 1.—Characteristics of patients and tumors

Characteristic	No. of patients (%)
Age (yrs)	
< 50	16 (25)
50–59	25 (38)
60–69	19 (29)
≥ 70	6 (9)
Race	
White	57 (84)
Nonwhite	11
Sex	
Male	46 (68)
Female	22
Location of primary tumor	
Head	39
Body/tail	26
Grade of anaplasia	
High	38 (58)
Low	28
Prior chemotherapy	
No	36 (53)
Yes	32
Initial performance status*	
0,1	23
2,3	39

*ECOG scale: 0 (fully ambulatory) to 4 (totally disabled).

RESULTS

A total of 82 patients were entered into the study by 25 contributing institutions. Of this group, 14 were declared ineligible: eight because they did not have the required measurable lesion, three because the primary tumor was not pancreatic adenocarcinoma, two because there was no histologic confirmation, and one because therapy was initiated only 5 days after abdominal surgery. All other eligible patients who were given initial treatment are included in the results. Specifically, we did not exclude patients because of early death.

Toxicity

The only toxic reactions observed were nausea and vomiting (on the day of treatment) and hematologic depression. The occurrence of leukopenia, thrombocytopenia, and anemia among patients with adequately documented post-treatment data is shown in table 2. In this tabulation we have also included the toxicity experience of patients declared ineligible on the basis of the lack of an acceptable indicator lesion. Among 44 patients with adequately recorded counts, 35 (80%) had either leukopenia, thrombocytopenia, or both. The median time to nadir was 29 days for leukopenia and 22 days for thrombocytopenia. Hematologic toxicity was severe in several patients, but there was only one drug-related death.

Therapeutic Effect

Among 68 patients eligible and evaluable for therapeutic response, only four (6%) demonstrated objective improvement (table 3). One patient had a complete response persisting for 22 months after the beginning of therapy, and three patients had partial responses for 7, 14, and 21 weeks. Two of the re-

TABLE 2.—Hematologic toxicity to initial course of methyl-CCNU therapy

Toxic reaction	Frequency*
Leukopenia (cells/mm^3)	
< 4000 > 2000	16/44 (36)
< 2000	19/44 (43)
Thrombocytopenia (platelets/mm^3)	
< 150,000 > 100,000	6/41 (15)
< 100,000 > 25,000	22/41 (53)
≤ 25,000	5/41 (12)
Anemia (decrease in hemoglobin > 2.0 g/100 ml)	20/49 (41)

*No. of patients with toxic reaction/total No. of patients (%).

TABLE 3.—Therapeutic results of methyl-CCNU therapy in 66 eligible and evaluable patients

Objective result	No. of patients with—		Total No. of patients (%)
	No prior chemotherapy	Prior chemotherapy	
Improved	3 (8)	1 (3)	4 (6)
Stable	8	6	14
Progression	25	25	50
Totals	36	32	68

sponding patients had had no prior chemotherapy and two had been previously treated. Fourteen patients remained objectively stable over periods ranging from 7 to 21 weeks. Fifty patients showed objective progression at 7 weeks. The median survival of the 68 eligible patients was 8 weeks from the onset of therapy with a range of from 3 days to 33½ months.

DISCUSSION

The objective response rate of only 6% recorded in this study among 68 patients treated with methyl-CCNU is essentially the same as the 5% response rate among 56 patients treated with CCNU and BCNU as culled from the literature by Comis and Carter (2). It is evident that none of the currently available nitrosoureas, used alone at maximum tolerable doses, provide any significant benefit to patients with advanced pancreatic carcinoma.

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Some Cardiovascular Effects of Cyclocytidine [1,2,3]

E. H. Herman [4,5,*] and D. P. Chadwick [4,6]

Brief Reports and Preliminary Communications

Cytosine arabinoside (Ara C)[7] has been widely used in the clinical treatment of acute myelogenous and acute lymphocytic leukemia (1). Cyclocytidine is an anhydride of Ara C and this modification alters the properties of the molecule to such an extent that other significant pharmacologic actions appear. Thus, in addition to antineoplastic activity, cyclocytidine has been reported to cause sialorrhea, parotid pain, and postural hypotension in patients (2). None of these side effects has been observed previously during Ara C therapy. Experimentally, the sialogogic actions of cyclocytidine have been examined in rat salivary glands (3). Some cardiovascular actions have been reported in certain anesthetized animals (4). The present experiments were initiated to further assess the cardiovascular actions of cyclocytidine.

MATERIALS AND METHODS

Intact Anesthetized Monkey Preparation

Male rhesus monkeys (weight, 3.5–4.5 kg) were anesthetized with 30 mg/kg of pentobarbital sodium (iv) and prepared for recording of arterial blood pressure, cardiac output, left ventricular force of contraction, heart rate, and lead II electrocardiogram as previously described (5). All parameters were monitored on a Hewlett-Packard polygraph recorder. At least 30 minutes of stable recordings were obtained prior to the beginning of any experiment.

Groups of four animals each were given iv doses of 50 or 100 mg/kg of cyclocytidine hydrochloride. The compound was dissolved in 0.9% physiologic saline and infused over a 4-minute period.

A series of four animals was given 0.5 mg/kg of dl-propranolol 15 minutes prior to the injection of 100 mg/kg (three animals) or 50 mg/kg of cyclocytidine (one animal). The effect of cyclocytidine and propranolol pretreatment on cyclocytidine was evaluated over a 60-minute period.

Isolated Dog Heart Preparation

The direct cardiac actions of cyclocytidine were studied in 15 isolated blood-perfused dog heart preparations. This preparation, which monitors force of contraction (left ventricular), electrocardiogram, heart rate, and pump perfusion pressure (reflection of coronary arterial pressure), has been previously described in detail (6). Three hearts each were given either 2.5, 5.0, or 10.0 mg of cyclocytidine dissolved in 5 ml of 0.9% physiologic saline directly into the approximately 500-ml perfusion circuit.

A series of three isolated hearts was pretreated with 0.5 mg of propranolol 10 minutes prior to the administration of 10 mg of cyclocytidine.

In a second series, three dogs were pretreated with 0.1 mg/kg of reserpine (im) on 2 consecutive days. Hearts from these animals were utilized 24 hours after the second dose of reserpine. Each heart was given 10 mg of cyclocytidine. All experiments were terminated 60 minutes after cyclocytidine administration.

RESULTS

Intact Anesthetized Monkey

In general, mean arterial pressure, heart rate, aortic blood flow, and left ventricular force of contraction all increased approximately 20% immediately after administration of 100 mg/kg of cyclocyti-

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[2]Supported by contract N01-CM-33728 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Cyclocytidine: NSC-145668; CAS reg. No. 10212-25-6; 6*H*-furo[2′,3′:4,5]oxazolo[3,2-*a*]pyrimidine-2-methanol, 2,3,3*a*,9*a*-tetrahydro-3-hydroxy-6-imino-, monohydrochloride. Obtained from the Developmental Therapeutics Program, DCT, NCI.

[4]Microbiological Associates, Inc, Bethesda, Md.

[5]*Present address:* Bureau of Drugs, Food and Drug Administration (FDA), Washington, DC.

[6]*Present address:* Bureau of Biologics, FDA, Bethesda, Md.

[7]Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride.

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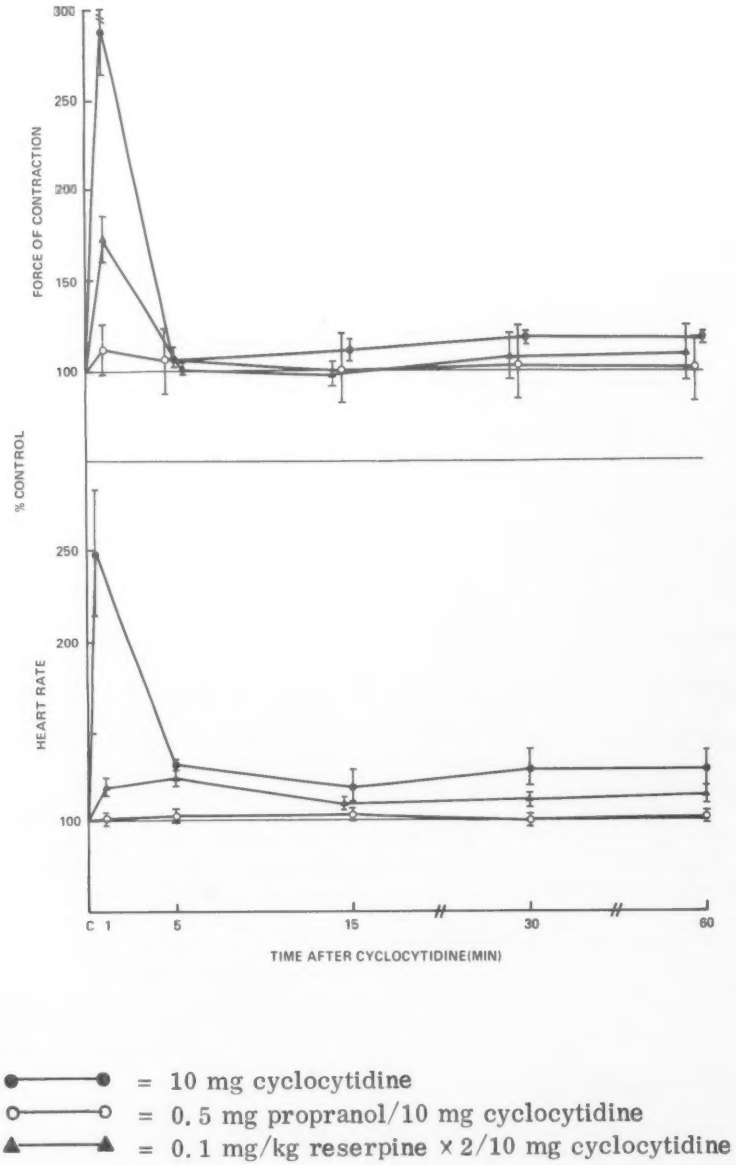

FIGURE 1.—Influence of pretreatment with propranolol (0.5 mg) or reserpine (0.1 mg/kg × 2) on the positive inotropic and chronotropic responses of 10 mg of cyclocytidine in the isolated blood-perfused dog heart preparation. Values obtained were converted to % of control with each preparation serving as its own control. Each point represents the mean ± SE from 3 separate experiments.

dine. Although some recovery was noted, all parameters were still above control levels 60 minutes after injection.

A dose of 50 mg/kg of cyclocytidine produced effects similar to those described for the 100-mg/kg dose with certain exceptions. The magnitude of the increases in the various cardiovascular parameters was less (10%–15%) and in most experiments the increases were reversible within 30–60 minutes.

The administration of 0.5 mg/kg of propranolol decreased the various cardiovascular parameters between 8% and 25% below control levels. This dose of propranolol prevented the increases in aortic blood flow, heart rate, left ventricular contraction, and pulse pressure seen previously after 100 mg/kg of cyclocytidine. However, the increase in mean arterial pressure was comparable in both treated and nontreated animals. Similar effects were noted in one animal pretreated with propranolol followed by 50 mg/kg of cyclocytidine.

Isolated Dog Heart Preparation

Cyclocytidine (2.5–10.0 mg) caused a dose-related increase in force of contraction (190%–290%) which reached maximal intensity within 1 minute and thereafter rapidly returned toward control levels. In addition, all doses increased the heart rate within 1 minute (125%–250%) followed by a rapid return toward control values.

Coronary perfusion pressure was decreased between 10% and 50%. A partial recovery in this parameter was noted in most experiments during the 60-minute experimental period.

The effects of pretreatment with reserpine or propranolol on heart rate and force of contraction after 10 mg of cyclocytidine are summarized in figure 1. Reserpine significantly attenuated both responses. However, the positive inotropic and chronotropic actions of cyclocytidine were almost completely eliminated by pretreatment with propranolol.

DISCUSSION

Cyclocytidine caused immediate and sustained cardiovascular alterations in the rhesus monkey. The compound increased functions such as aortic blood flow, left ventricular force of contraction, heart rate, pulse pressure, and arterial blood pressure. Bolus injections of cyclocytidine have also been found to elevate blood pressure in anesthetized dogs, cats, and rats (4). In the present experiments, pretreatment with propranolol blocked all the effects of cyclocytidine except the increase in blood pressure. The increase in blood pressure could occur in spite of propranolol pretreatment if cyclocytidine increased vascular tone through an α-adrenergic mechanism. Such a mechanism appears operative since the hypertensive response has been attenuated by phentolamine (4).

Some of these actions might be explained by observations in the isolated heart experiments. In these studies, all doses of cyclocytidine caused an immediate increase in both force of contraction and heart rate. Pretreatment with a low dose of reserpine modified the stimulatory effects of cyclocytidine, suggesting that initially the responses are at least in part mediated by a release of endogenous catecholamines. This type of effect may also explain the brief duration of action noted in isolated heart experiments. However, a direct sympathomimetic action may also be involved since cyclocytidine still elicited positive inotropic and chronotropic responses in reserpinized hearts. In either case, all cardiac-stimulant actions of cyclocytidine in the isolated heart were completely blocked by pretreatment with propranolol. The results tend to suggest that the actions of cyclocytidine are mediated directly and indirectly through β receptors in the heart and a similar action in the intact animal might be responsible for some of the observed initial cardiovascular effects of cyclocytidine.

During the course of these experiments, cyclocytidine induced only sympathomimetic activity while clinically postural hypotension has been reported. Similar effects occur with agents such as guanethidine, where initially single-dose administration produces an immediate period of adrenergic-like stimulation (7,8). This initial response appears to be due to the release of catecholamines since it is almost entirely eliminated by pretreatment with agents such as reserpine or imipramine (8,9). Ultimately, guanethidine induces postural hypotension by altering sympathetic vascular control through an action on sympathetic nerve terminals (7). An alteration in sympathetic vascular control similar to that elicited by guanethidine could explain the postural hypotension reported after clinical use of cyclocytidine.

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Evaluation of Daunorubicin in Adenocarcinoma of the Large Intestine[1,2,3]

Arthur J. Weiss[4,5,*] and Ronald I. Cantor[4,5]

The anthracycline group of antitumor antibiotics includes daunorubicin and its hydroxylated derivative, adriamycin (1,2).[6] Adriamycin has been studied extensively in many types of malignancies and is recognized as having a broad antitumor spectrum; it is effective against leukemias, lymphomas, and many solid tumors including carcinoma of the breast, lung, and various soft tissue sarcomas (3–7). In the original reports of Bonadonna et al, an appreciable but transient objective remission rate was reported for adenocarcinoma of the large bowel (3). It is generally accepted, however, that adriamycin is essentially ineffective in this neoplasm; remission rates of ≤ 7% have been reported in most series (6). Daunorubicin has been studied in lymphomas and leukemias and also, to a limited extent, in various solid tumors. It is believed that its activity is roughly comparable to adriamycin against lymphomas and leukemias and that it is less effective than adriamycin in most solid tumors (7,8). Evans et al (9) described a controlled trial in which daunorubicin was compared to adriamycin in pediatric solid tumors. In this study the activity of both agents was roughly comparable.

Because of this, it was felt that a pilot study to determine whether daunorubicin is effective against a solid tumor known to be resistant to adriamycin would be worthwhile. Adenocarcinoma of the large intestine was selected as an appropriate tumor to study.

MATERIAL AND METHODS

Only patients who had biopsy-proven adenocarcinoma of the colon or rectum with evidence of metastatic disease were chosen for study. All patients had to have measurable lesions. Grossly abnormal liver scans with discrete defects were acceptable for measurement provided biopsy proof of hepatic metastatic disease already existed. The wbc count had to be ≥ 4000 cells/mm^3 and the platelet count ≥ 125,000 platelets/mm^3. Renal function as measured by BUN and creatinine serum levels had to be within normal limits. While significant hepatic dysfunction was acceptable, no patient was started on study whose total serum bilirubin was ≥ 2.5 mg/100 ml. Hematologic studies were obtained weekly and hepatic and renal studies every 2–4 weeks. Soft tissue and pulmonary lesions were measured every second week. Hepatic scans were repeated every 6–8 weeks. Electrocardiograms and clinical cardiac evaluations were performed monthly. The treatment schedule which was followed is presented in figure 1.

RESULTS AND DISCUSSION

The toxicity which was seen is detailed in table 1. All patients exhibited evidence of marrow suppression as manifested by leukopenia. Thrombocytopenia was seen in approximately 70% of the patients treated. Stomatitis occurred in approximately 50% of the patients; occasionally this was quite severe but it never lasted > 2 weeks after the drug was stopped. Diarrhea was noted in seven patients; however, in each case it was believed to be due primarily to the patient's disease and not the drug. One patient developed an arrhythmia (paroxysmal atrial tachycardia) soon after daunorubicin was started. This was controlled with digitalis, and daunorubicin was continued without difficulty. Another patient developed ventricular extrasystoles that persisted during therapy with daunorubicin. There was no other evidence of cardiac toxicity.

The remissions seen are described in table 2. Patients were considered as having an objective remission if they had a decrease of ≥ 50% in the mean area of all lesions being measured for a minimum period of 8 weeks. (The area of each lesion was approximated as being proportional to the product of the largest diameter of the lesion times its perpendicular.) Subjective deterioration in the con-

[1]Received May 17, 1976; revised Oct 4, 1976; accepted Oct 8, 1976.

[2]Daunorubicin: NSC-82151; CAS reg. No. 23541-50-6.

[3]This study was conducted as a pilot study by the Central Oncology Group.

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[6]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

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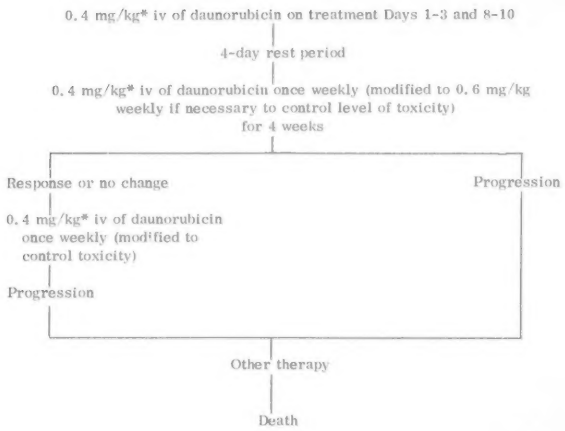
0.4 mg/kg* iv of daunorubicin on treatment Days 1-3 and 8-10
4-day rest period
0.4 mg/kg* iv of daunorubicin once weekly (modified to 0.6 mg/kg weekly if necessary to control level of toxicity) for 4 weeks
Response or no change
Progression
0.4 mg/kg* iv of daunorubicin once weekly (modified to control toxicity)
Progression
Other therapy
Death

Toxicity	Suggested therapy change
Wbc count: < 3000 cells/mm^3	Discontinue therapy with daunorubicin temporarily
Platelet count: < 80,000 cells/mm^3	Discontinue therapy with daunorubicin temporarily
CPK level: 150% of upper limit of normal range	Discontinue therapy with daunorubicin
SGOT level: 150% of initial value and abnormal	If not caused by disease, discontinue drug temporarily
Bilirubin level: 150% of initial value and abnormal	If not caused by disease, discontinue drug temporarily
EKG: changes of arrhythmia, infarction, ischemia, pericarditis, or other cardiac abnormality	Discontinue therapy with daunorubicin temporarily
Stomatitis: mild	Withhold next drug dose; increase interval between doses by 50%; follow wbc and platelet counts closely
Stomatitis: severe	Discontinue therapy with daunorubicin temporarily

*Based on the lesser of actual and ideal body weights.

FIGURE 1.—Daunorubicin therapy in adenocarcinoma of the large bowel.

dition of the patient prevented that patient from being classified as having an objective remission. Of the 57 patients we studied, six had evidence of an objective remission for a remission rate of 10.5%. While this remission rate is quite low, it approximates that found in some series using 5-fluorouracil (5-FU) (10). In four patients, the character of the remission was quite gratifying; one patient remained in remission for 2 years, another for 16 months, and two patients for 12 months.

The return of abnormal liver function to normal was not unusual in those patients who had remissions. Four of the remissions were seen in patients with quite advanced disease. Two of the remissions were noted before any evidence of gastrointestinal or hematologic toxicity was seen. Subjective improvement (when it occurred) was quite dramatic and all patients that improved objectively showed moderate-to-marked subjective benefit.

Seventy percent of the patients in this study had evidence of hepatic metastatic disease. All the patients who had remission had hepatic metastatic disease, and it was our impression that hepatic metastases seemed to respond more rapidly and

TABLE 1.—Summary of toxicity

Toxic effect	% of patients with degree of severity of—*					% of patients with abnormal hemoglobin or hematocrit level	Median treatment day of greatest severity
	0	1	2	3	4		
Nausea and vomiting	51	33	14	2	0		5
Diarrhea	75	11	12	2	0		12
Stomatitis	6	37	37	16	4		18
Alopecia	42	11	26	21	0		44
Leukopenia	0	0	79	16	4		26
Thrombocytopenia	4	21	68	6	0		33
Anemia†						72	74

*Nausea and vomiting, diarrhea, and stomatitis: 0 = none; 1 = mild; 2 = moderate; 3 = severe (required parenteral supplementation); 4 = life threatening. Alopecia: 0 = none; 1 = mild; 2 = moderate; 3 = almost complete loss of scalp or body hair; 4 = total loss of all hair. Leukopenia (cells/mm³): 0 = ≥ 5000; 1 = 4000–4999; 2 = 3000–3999; 3 = 2000–2999; 4 = < 2000. Thrombocytopenia (platelets/mm³): 0 = ≥ 150,000; 1 = 125,000–149,999; 2 = 75,000–124,999; 3 = 50,000–74,999; 4 = < 50,000.

†Abnormal hemoglobin = < 10 g/100 ml and abnormal hematocrit = < 30% due to either drug or disease.

TABLE 2.—Objective response rates by primary tumor site

Disease site	Total dose (mg/m²)	No. of patients evaluable	No. of patients with—			Remission duration (mos)
			Objective response	No change	Progression	
Cecum and right colon	248	5	1	1	3	2.5
Transverse and sigmoid colon	465	21	3	11	7	5.0
	652					16.5
	526					12.0
Rectosigmoid and rectum	850	31	2	14	15	24.0
	550					12.0

completely than metastases at other sites. All of the patients that had remissions were men; however, 70% of the patients in this series were men.

All of the patients in this series had received prior therapy with 5-FU and, at the time daunorubicin was studied, were resistant to this agent. Four of the patients who responded to daunorubicin were initially resistant to 5-FU; two of the responders had previously responded to 5-FU and relapsed. None of the patients had been previously treated with adriamycin.

While the number of patients treated in this series was quite small and the objective remission rate leaves much to be desired, our results must be considered in light of advanced 5-FU-resistant disease. If one excludes previously untreated patients from the Frytak et al (6) series, their response rate drops to < 3%. So not only was our response rate more than triple theirs, but our median duration of response (12 months) was much better than theirs (3 months). It is our feeling that daunorubicin has demonstrated a limited but definite activity against metastatic adenocarcinomas arising in the colorectum. Further trials with daunorubicin used either alone or in combination with other agents in this disease and perhaps in certain other neoplasms of the gastrointestinal tract seem to be warranted.

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Dihydro-5-Azacytidine Hydrochloride, a Biologically Active and Chemically Stable Analog of 5-Azacytidine[1,2]

Brief Reports and Preliminary Communications

John A. Beisler,[3,*] Mohamed M. Abbasi,[3,4] and John S. Driscoll [3]

5-Azacytidine (5-AC) is a pyrimidine nucleoside antimetabolite which has secured a place among the antineoplastic drugs (1).[5] Although 5-AC is apparently of limited value in the clinical treatment of solid tumors,[5] it has been effective in the treatment of acute leukemias (2,3), exhibiting a specificity against acute myelogenous leukemia (2,3) as indicated by a 36% overall response rate.[5] The major dose-limiting toxicity, when administered either iv or sc as a bolus dose, has been severe nausea and vomiting (4,5) which has caused patients to refuse further courses of treatment. This toxicity has been mitigated by administration of the drug in divided doses (2,5) or by slow iv infusion (6,7). A study by Lomen et al (8), using a technique of continuous infusion of the drug over a 5-day period, effected the virtual elimination of nausea and vomiting while still preserving clinical activity. However, 5-AC decomposes quite readily in water or aqueous formulations (7,9,10)[5] giving products of unknown toxicity and making strict control over dosages very difficult at the clinical level using a continuous-infusion technique.

We would like to report the synthesis of a 5-AC analog, which is stable for weeks at room temperature in aqueous solution over a broad pH range and which has a substantial and reproducible activity in the mouse L1210 leukemia system.

CHEMISTRY

An appraisal of the decomposition mechanism of 5-AC in aqueous solution (9,10) prompted us to consider a reduction of the 5,6 double bond as a means to increase the stability of the drug toward hydrolysis. After fulfilling its stabilizing function, the reduced nucleoside could conceivably be reoxidized to 5-AC in vivo. If this oxidation occurred, the reduced nucleoside could be considered a pro-drug (11) of 5-AC, and as such the clinical experience with 5-AC might be generally applicable to the reduced form also.

The reduction of 5-AC was accomplished with sodium borohydride in hexamethylphosphoramide solution to give the reduced nucleoside as a boron complex. Hydrolysis of the complex with hydrochloric acid yielded 5,6-dihydro-5-azacytidine as the hydrochloride salt (5-AC[H]), melting point (mp) 180°C–181°C (decomposition), λ_{max}(pH 8) 233 nm (ϵ = 4980). The nuclear magnetic resonance (NMR) spectrum (DMSO-d_6) showed the absence of the aromatic proton (σ 8.55) characteristic of 5-AC and the presence of a singlet at σ 4.69 (two protons) due to the C-6 methylene group of 5-AC[H]. Treatment of 5-AC[H] as the free base with bis-(trimethylsilyl)-trifluoroacetamide in acetonitrile solution for 10 minutes at room temperature provided a volatile *pentakis*-trimethylsilyl derivative which gave a molecular ion at m/e 606 in the mass spectrum.[6] Using the same trimethylsilylation procedure 5-AC formed a *tetrakis*-trimethylsilyl derivative which gave a molecular ion at m/e 532. The structures of 5-AC and 5-AC[H] are shown for comparison in figure 1.

ANALYTIC PROCEDURES

Hydrolysis Stability

The solution stability of 5-AC[H] at pH 2 and 6 was followed over a 3-week period using an NMR method for analysis. Accordingly, a 0.18 M solution of 5-AC[H] containing 0.1 M KCl gave a solution (pH

[1]Received May 7, 1976; revised Sept 15, 1976; accepted Sept 22, 1976.

[2]5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; *s*-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-.

Dihydro-5-azacytidine hydrochloride: NSC-264880; 1,3,5-triazin-2(1*H*)-one, 4-amino-5,6-dihydro-1-β-D-ribofuranoyl-, monohydrochloride.

[3]Laboratory of Medicinal Chemistry and Biology (LMCB), Developmental Therapeutics Program (DTP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

[4]National Institutes of Health (NIH) Visiting Fellow.

[5]Von Hoff DD, Handelsman H, and Slavik M. Clinical brochure: 5-azacytidine (NSC-102816). Prepared by the DCT, NCI, Bethesda, Md, Aug 1975, 107 pp.

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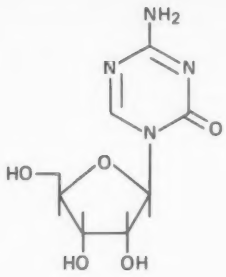
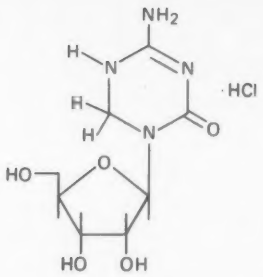
[6]Spectroscopic, analytic, and chemical data in support of the structure assigned to 5-AC[H] will be reported in detail at a later date.



5 – Azacytidine
(NSC 102816)



Dihydro-5-azacytidine hydrochloride
(NSC 264880)

FIGURE 1.—Structures of 5-AC and 5-AC[H].

2.0) which was lyophilized and dissolved in D_2O. Lyophilization and dissolution in D_2O were repeated several times to completely exchange labile protons. The final lyophilizate was dissolved in the volume of 100% D_2O necessary to make a solution having the initial concentrations of 5-AC[H] and KCl.

In a similar way, a second sample of 5-AC[H] was prepared (pH 6.0) with phosphate buffer, exchanged with deuterium, and dissolved in 100% D_2O affording a solution 0.17 M in 5-AC[H] and 0.05 M each in KH_2PO_4 and Na_2HPO_4. Integrated NMR[7] spectra of each solution were recorded immediately and at regular intervals thereafter, using an internal standard of $CHCl_3$-CCl_4(1:1) sealed in 1-mm ID glass capillary tubes. Between determinations, the NMR-sample tubes were stored at 24°C–25°C and at normal room illumination. Over the 3-week observation period no change in either sample was noted as demonstrated by a comparison of the integrated areas of the internal standard with the ribosyl anomeric proton, the methylene protons of the triazine ring, and the remaining nonexchangeable protons of the ribose moiety. Although 5-AC[H] has a water solubility of > 200 mg/ml, the free base is soluble in water to the extent of about 16 mg/ml. Therefore, it was not practical with our spectrometer to extend the analysis of 5-AC[H] to solutions having higher pH values. However, a solution of 5-AC[H] in 1 N NH_4OH (pH 11.8) was stored at 25°C for 7 days and showed no decomposition evidence (thin-layer chromatography). The free base of 5-AC[H] (mp 218°C–220°C) (NSC-265483) was isolated from the solution in good yield and purity. By contrast, a solution of 5-AC in 1 N NH_4OH would be completely decomposed in several hours (9).

[7]Varian Associates, Model T-60 spectrometer.

Drug Purity

Samples of 5-AC[H] contaminated with as little as 1%–3% of 5-AC, which could conceivably be carried as an impurity through the synthetic sequence, might exhibit an antitumor effect that arises solely from the 5-AC contamination. It was, therefore, essential to demonstrate the absence of 5-AC in our 5-AC[H] samples. A high-pressure liquid chromatograph[8] (HPLC) equipped with a fixed wavelength (254 nm) ultraviolet (UV) detector was well suited to provide the necessary analytic data at the required sensitivity. The UV detector was shown to give a linear response when calibrated with aqueous 5-AC solutions of 0.05, 0.035, 0.02, and 0.005 mg/ml. The 5-AC peak (retention time: 3.2 minutes) produced by 20-μl injections of the calibration solutions gave mean detector responses in absorbance units[9] of 0.0401(2), 0.0271(7), 0.0158(2), and 0.0038(5) respectively. A 10-mg/ml solution of 5-AC[H] in water when injected (20 μl) into the HPLC gave a chromatogram which showed no deviation from the base line. By contrast, an aqueous solution of 5-AC[H] (10 mg/ml) which had been artificially contaminated with 0.05% 5-AC gave a clearly defined peak having an absorbance of 0.0042 units with a retention time of 3.2 minutes. Additional samples of 5-AC[H] artificially contaminated with 0.2% and 0.125% 5-AC similarly gave the anticipated detector response. Since 5-AC[H] does not absorb in the UV at 254 nm, it is not observable on the chromatogram and does not interfere with the analysis for 5-AC. However, 5-AC[H] can be observed in the void vol-

[8]Waters Model ALC/GPC-244 fitted with a 1/4 × 12-inch μBondapak/C_{18} column and eluted at 2 ml/min with water.

[9]Mean deviations, given in parentheses, refer to the last decimal position.

TABLE 1.—Comparison of 5-AC[H] and 5-AC against L1210 leukemia (q4d, 0.9% saline vehicle)

5-AC[H]					5-AC			
Dose (mg/kg)	%ILS for control No.—				Dose (mg/kg)	%ILS for control No.—		
	8884	8887	8895a	8895b		8884	8887	8895
800			96		40	toxic	22	09
600		104		83	20	81	138	74
500		81			10	144	93	94
400	62	75	80		5	58	64	68
300		64		82				
200	49		72					
150		64		74				
100	51		61					
75				47				
50	35		52					
37.5				41				
25	30		27					
18.7				31				
12.5	29		23					
6.25	07							

ume (1.2 minutes) of the chromatogram when the refractive index detector is used.

It can be concluded that 5-AC[H] contains no detectable amount of 5-AC. If 5-AC is present at all in our samples of 5-AC[H], it is present at concentrations considerably < 0.05% and would not influence the results of the antitumor assays.

BIOLOGIC EVALUATION

The L1210 leukemia system was used to evaluate the antitumor activity of 5-AC[H] according to protocols (12) devised by the DCT, NCI, wherein tumor cells are inoculated ip and drug is administered ip. Evaluation of 5-AC[H] was carried out using a Day 1, 5, and 9 treatment schedule. A control group of tumored mice treated with 5-AC was routinely run in parallel with each 5-AC[H] test. The results of these tests are recorded in table 1 as increases in lifespan (ILS) over untreated controls.[10]

DISCUSSION

It can be seen from table 1 that 5-AC[H] has comparable antitumor activity with 5-AC in the L1210 assay although the dose levels of 5-AC[H] required to produce the maximum response are much higher than the optimal doses of 5-AC. Evaluation of 5-AC[H] is continuing with the L1210 system using other treatment schedules and also with different murine tumor systems. As part of our initial efforts to test the hypothesis that 5-AC[H] acts as a pro-drug form of 5-AC, an L1210 subline, resistant to 5-AC, was used in a parallel experiment[11] with 5-AC and 5-AC[H]. Both 5-AC and 5-AC[H] were completely inactive against the resistant subline at dose levels which were therapeutic against the L1210 cell line used to obtain the data in table 1. The observed cross-resistance could be explained by an in vivo conversion of 5-AC[H] to 5-AC, but more conclusive evidence must await metabolite studies.

If the necessary higher dose levels of the reduced nucleoside prove to be not disadvantageous, we believe 5-AC[H] would be a strong candidate for further comparative preclinical studies. It has excellent stability in water solutions over a broad pH range, has good water solubility for formulation ease, and has L1210 leukemia activity comparable to that of 5-AC.

If 5-AC[H] also induces nausea and vomiting when administered as a bolus, administration of the drug by the continuous-infusion technique would offer no difficulty due to drug instability.

[10]Antitumor evaluations were provided by L. Dudeck and G. Congleton, Hazleton Laboratories, Vienna, Va.

[11]This assay was provided by I. Wodinsky, Arthur D. Little, Inc, Cambridge, Mass.

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Comparative Nephrotoxicity of Platinum Cancer Chemotherapeutic Agents[1]

Brief Reports and Preliminary Communications

J. M. Ward,* D. M. Young, K. A. Fauvie, M. K. Wolpert, R. Davis, and A. M. Guarino[2,3]

A new class of cancer chemotherapeutic drugs containing platinum is being developed, with *cis*-dichlorodiammineplatinum(II) [*cis*-DDP(II)][4] as the prototype (1–3). The toxic effects of *cis*-DDP(II) in man, dogs, monkeys, and rats involved the hematopoietic, otic, intestinal, lymphoid, and renal organ systems (4–8). The dose-limiting toxicity after multiple-drug administration in man and rats involves the kidney (5,9). After a series of multiple injections, humans and rats had continuing evidence of renal malfunction. In order to further develop analogs of *cis*-DDP(II) containing platinum and hopefully to find some with less renal toxicity than produced by the parent compound, many additional drugs containing platinum have been synthesized. The purpose of this study was to identify nephrotoxic platinum drugs and to characterize the nephropathology induced by the drugs.

MATERIALS AND METHODS

The drugs were supplied by the Developmental Therapeutics Program, Division of Cancer Treatment, NCI. Chloroplatinic acid (NSC-4958) was obtained commercially (J. T. Baker Chemical Co, Phillipsburg, NJ). The solubility of the drugs in saline or dextrose was variable; several required heating at 40°C for ≤ 1 hour in order to dissolve in the vehicle.

Four-week-old male F344 (Fischer, CDF) rats were obtained from the Charles River Breeding Laboratories, Wilmington, Mass. They were placed in methacrylate cages, fed NIH mouse and rat ration, and given water ad libitum. At 6 weeks of age they were injected ip or iv with the drugs.

The total amount of most drugs was limited; usually, only 1–2 g could be obtained. Therefore, LD50 (single injection, 14-day observation) studies could only be performed for two drugs using groups of ten rats for each dose (10,11). For *cis*-DDP(II) 80 rats were injected iv at the LD50 dose level (7.4 mg/kg) and groups of five rats were killed on Days 1–5, 7, 10, and 14. Initial toxicity studies for the other four drugs involved injections of two rats with one of five different doses of one of the four drugs. Additional studies were performed utilizing approximate LD50 doses and the rats were killed from 3 to 14 days after drug administration. The rats were weighed daily, the day of death was recorded, and the surviving rats were killed 14 days after injection. Rats were anesthetized with ether, blood was obtained from the abdominal aorta, and serum was prepared for determination of BUN, serum creatinine, uric acid, and SGPT levels (12–14). A complete necropsy was performed on each rat and tissues were placed in Telly's fixative, embedded in paraffin, sectioned at 5μ-6μ, and stained with hematoxylin and eosin. Selected tissues were stained by the Von Kossa and periodic acid-Schiff methods. The type and severity of renal lesions were recorded. The distal femur was placed in Telly's fixative and demineralized in EDTA, and the cellularity of the bone marrow was noted.

RESULTS

Cis-DDP(II)

The LD50 (iv injection, 14-day observation) of *cis*-DDP(II) was 7.4 mg/kg (95% confidence limits, 7.2–7.7 mg/kg). The LD10 was 6.8 mg/kg (6.5–7.3 mg/kg) and the LD90 was 8.1 mg/kg (7.7–8.5 mg/kg). After an iv LD50 dose, the mean BUN and serum creatinine levels increased, reached a peak level on Days 4–5, and returned to near normal by Day 14 when all surviving rats were gaining weight (fig 1).

Rats receiving LD50 doses iv developed diarrhea by Day 3 and died from Days 3 to 6. As previously described (9) histopathologic evaluation of various tissues (table 1) revealed necrotizing enteritis (small

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[2]Laboratory of Toxicology (LT) (Drs. Ward, Young, and Guarino, Mr. Faurie, and R. Davis) and Drug Evaluation Branch (Dr. Wolpert), Developmental Therapeutics Program (DTP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

[3]We would like to acknowledge the excellent assistance of Snooky Taylor, Kenneth K. Pavkov (Battelle Toxicology Program Office), Myra Grabin, and Madie Tyler.

[4]*Cis*-dichlorodiammineplatinum(II): NSC-119875; CAS reg. No. 15663-27-1.

**Reprint requests to:* Dr. J. M. Ward, LT, DTP, DCT, NCI, Bldg 37, Rm 5B22, National Institutes of Health, Bethesda, Md 20014.

TABLE 1.—Summary of lethal doses and histologic lesions in rats receiving platinum drugs*

Drug (NSC No.)	Route of administration	LD50 (mg/kg)	Lesions (degree of severity)†					
			Kidney	Peritoneal cavity	Liver	Intestine	Bone marrow	Thymus
Cis-DDP(II)	ip	7.6	3	0	0	3	3	3
	iv	7.4	3	0	0	3	3	3
250427	ip	7.0–8.0‡	1	3	1	3	2	3
	iv	8.8	3	0	3	1	3	3
239851	ip	7.0–8.0‡	1	3	1	3	2	3
	iv	8.0–9.0‡	3	0	3	0	2	3
Platinum uracil blue	ip	175–225‡	3	1	0	0	1	3
224964	ip	50–60‡	2	2	1	3	3	3
Chloroplatinic acid	ip	40–50‡	3	1	0	0	0	3

*The most advanced lesions detected at any dose level (which was usually the highest dose level administered).
†0 = absence of lesions; 1 = mild lesions or lesions in a small percentage of rats only; 2 = moderate lesions; 3 = severe lesions.
‡Estimated.

intestine, cecum, and colon), cellular depletion of bone marrow and thymus, and acute degenerative and necrotizing renal tubular lesions in the proximal tubules of the outer stripe (pars recta) of the renal medulla. These lesions in part or collectively were considered to be instrumental in the course of death of the rats. By Days 5–7 the surviving rats had regenerative renal tubular lesions in the pars recta which persisted to Day 14.

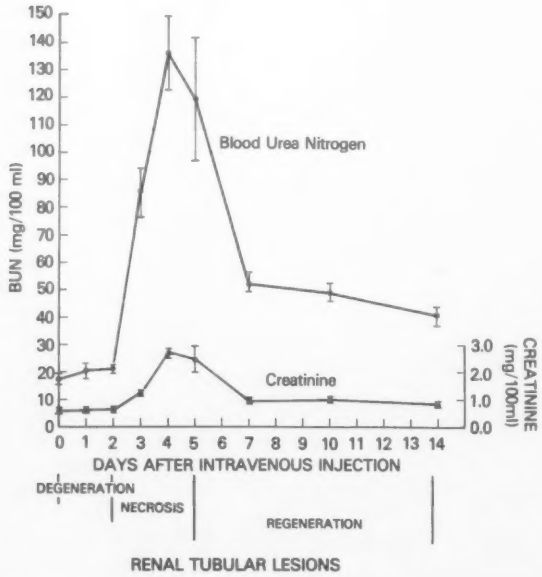

FIGURE 1.—Mean (± SE) BUN and serum creatinine levels in rats injected with *cis*-DDP(II) at a dose level of 7.4 mg/kg iv and killed in groups of 5 rats on Days 1–14. Degeneration, necrosis, and regeneration of proximal tubule cells in the pars recta parallel the changes in the BUN and creatinine levels.

NSC-250427 (Platinum, (1,2-cyclohexanediamine-*N*,*N*′)[sulfato(2-)-*O*,*O*′]-, (*SP*-4-2-)

The LD50 (iv injection, 14-day observation) was 8.8 mg/kg (95% confidence limits, 8.5–9.1 mg/kg). The LD10 was 8.0 mg/kg (7.6–8.5 mg/kg) and the LD90 was 9.5 (9.0–10.1 mg/kg). The LD50 value after ip injection was in the range of 7.6–8.0 mg/kg. The drug was mildly nephrotoxic when injected ip as indicated by only slightly elevated mean BUN values and only mild renal lesions. However, when the drug was injected iv there were marked nephrotoxic lesions and the BUN levels reached 140 mg/100 ml on Day 5. Peritonitis (as evidenced by peritoneal fluid accumulation), and hepatic, lymphoreticular, intestinal, and bone marrow lesions were induced by ip injection of the drug, while hepatic, renal, lymphoreticular, and bone marrow lesions were observed after iv injection (table 1). The LD50 (iv injection, 14-day observation) study revealed that rats receiving the two highest dose levels (15 and 12 mg/kg) of the drug died by 3–4 days with severe midzonal necrotizing hepatic lesions and necrotizing and degenerative renal tubular lesions. Rats receiving 9.5 mg/kg of the drug iv died at 5–9 days with severe necrotizing, renal tubular lesions, and mineralization of nearly the entire cortex. However, necrotizing hepatic lesions were not seen although there were focal areas of hepatocytes

with hydropic changes. Hemorrhagic gastric erosions were seen grossly and microscopically in most rats.

NSC-239851 (Platinum, (1,2-cyclohexanediamine-*N*,*N*′)bis(nitrato-*O*)-, (*SP*-4-2)-)

Ip administration of this agent produced peritonitis, necrotizing hepatic lesions, and bone marrow and thymic depletion with mild or no renal tubular lesions (table 1). The BUN was only mildly elevated after high toxic doses. Iv administration induced significantly elevated BUN values which reached > 100 mg/100 ml by Days 3–5 and marked necrotizing renal tubular lesions in the entire cortex and midzonal hepatic necrosis.

NSC-180512 (Platinum uracil blue; 2,4(1*H*,3*H*)-pyrimidinedione, platinum complex)

Platinum uracil blue was administered at the highest dosages compared with the other platinum drugs since it was less toxic than the other five platinum compounds. The drug was nephrotoxic by ip injection as determined by elevated BUN (≤ 100 mg/100 ml) and creatinine values and severe necrotizing renal tubular lesions with mineralization in the outer stripe of the renal medulla. The renal lesions appeared to be the primary cause of death (table 1) unlike *cis*-DDP(II), NSC-250427, and NSC-239851 which induced severe toxicity in several tissues.

NSC-224964 (Platinum, (1,2-cyclohexanediamine-*N*,*N*′)[propanedioato-(2-)-*O*,*O*′]-, (*SP*-4-2)-)

Only 800 mg of this drug was available. Because of its poor solubility (0.5 mg/ml) and relatively high lethal dose (50–75 mg/kg) a large volume (10–15 ml) had to be injected ip or sc. Elevated BUN (≤ 125 mg/100 ml) and creatinine values were associated with necrotizing renal tubular lesions in the outer renal cortex. Necrotizing intestinal lesions were severe and some rats had hepatic necrosis. A combination of these toxic effects appeared to cause death (table 1).

NSC-4958 (Chloroplatinic acid; platinate(2-), hexachloro-, dihydrogen, hexahydrate)

Chloroplatinic acid was a primary renal toxin and induced minimal lesions in other tissues (table 1). Rats died of renal failure with BUN values as high as 300 mg/100 ml by Day 3, hypocalcemia, and hyperkalemia. The tubular necrotizing lesions involved the entire renal cortex. Renal mineralization occurred by Day 5 and rats died in hypocalcemic tetany.

DISCUSSION

This study showed that all six platinum-containing drugs were nephrotoxic by ip and/or iv administration. Two drugs with minimal renal toxicity by ip administration were highly toxic to the kidney after iv injection. The nephrotoxicity of chloroplatinic acid verified the potent toxicity of platinum-containing compounds to renal tubules. The most severe renal toxins (chloroplatinic acid, NSC-239851, platinum uracil blue, and NSC-250427) resulted in uremia and renal failure as the cause of death and produced necrotizing lesions of the proximal tubules throughout the entire renal cortex. The less nephrotoxic drugs [*cis*-DDP(II) and NSC-224964] and lower doses of the more nephrotoxic drugs induced lesions in proximal tubules of the medullary outer stripe. Similar dose response and localized toxic effects have been reported for other nephrotoxic agents (15,16). Mineralization of necrotic renal lesions was usually followed by death. The results of these studies suggest that platinum compounds should be considered as potent renal toxins along with other heavy metals.

The correlation of the elevated BUN values and histopathologic lesions in the kidney is necessary to determine the type and degree of nephrotoxicity. Previous studies indicate that histopathologic evaluation of the kidney is the most sensitive method for the determination of nephrotoxicity (16). Our studies support this contention. All platinum agents induced degenerative necrotizing and regenerative lesions in the proximal tubules associated with changes in BUN and creatinine levels. The results of our work and that of others indicate that the Fischer rat provides a sensitive strain for testing potentially nephrotoxic drugs.

Platinum salts have been previously described as respiratory allergens in man (17). The identification of chloroplatinic acid as a severe renal toxin, similar to that of mercuric chloride, suggests that these important nephrotoxic effects of platinum cancer chemotherapeutic agents are not limited to these drugs.

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Phase II Evaluation of *Cis*-diamminedichloroplatinum(II) in Lung Cancer [1,2]

Brief Reports and Preliminary Communications

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Cis-diamminedichloroplatinum(II) (CACP) is a member of the first class of inorganic compounds to be evaluated for activity against human neoplasia. Phase I trials performed at a number of cancer treatment centers have established safe dosages and schedules for phase II trials (1–5).

When using either the 5-day-fractionated or the single-dose schedule, toxicity appears identical (1). The convenience of the single-dose schedule and the apparent lack of schedule-dependency in experimental systems encouraged us to investigate the activity of CACP when given iv at a dose of 75 mg/m^2 every 3 weeks to patients with lung cancer. This investigation demonstrated a very low order of activity for CACP in our patients.

MATERIALS AND METHODS

CACP was diluted to a concentration of 1 mg/ml and given as a rapid iv infusion. No effort was made to prehydrate the patients and no forced diuresis with mannitol was attempted.

Eligibility criteria included a measurable lesion in a patient with histologically verified lung cancer, an anticipated survival of ≥ 3 months, complete recovery from toxicity due to previous chemotherapy and/or radiotherapy, a patent urinary tract confirmed by iv pyelography, wbc count ≥ 4000 cells/μl, platelet count ≥ 150,000 platelets/μl, BUN level ≤ 20 mg/dl, and an endogenous creatinine clearance ≥ 75 ml/minute. Patients with stable disease or improvement continued to receive the drug at 3-week intervals. Each week, patients had cbc and platelet counts and measurements of serum electrolytes, BUN, serum creatinine, and serum uric acid. Before each course of therapy and at the conclusion of the study, a 24-hour endogenous creatinine clearance and audiometry were assessed.

Partial regression (PR) was defined as a reduction by 50% of the product of the largest diameter of each measurable lesion and its perpendicular.

RESULTS

The results of this investigation are summarized in table 1. Only one PR was identified. This occurred in a woman with adenocarcinoma who had received no previous chemotherapy. After seven courses of CACP and 5 1/2 months in remission she experienced tinnitus and declined further therapy. Audiography was unchanged, however, and only mild leukopenia was identified. Four patients had stable disease and they were given five, four, four, and three courses of CACP until progression was defined. One patient with small cell carcinoma and one with undifferentiated tumor died of progressive disease before receiving the second dose of CACP. These two patients should not be considered fully evaluable for response; however, it is unlikely that the second dose would have produced regression.

As in previous studies, the major dose-limiting side effects of CACP were nausea and vomiting. Four instances were characterized as severe and six as moderate. There was one instance of moderate diarrhea.

In two cases, the BUN rose to values > 21 mg/dl but < 40 mg/dl; recovery was complete in both instances.

The platelet count became 40,000 platelets/μl in one patient with a bone marrow biopsy and aspirate that were positive for tumor. In another patient, a platelet nadir of 64,000 platelets/μl was reached. Hemorrhagic complications did not occur in either patient. No significant wbc depression was identified.

DISCUSSION

Favorable objective responses in a number of tumor types were noted during phase I investiga-

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[2]*Cis*-diamminedichloroplatinum(II): NSC-119875; CAS reg. No. 15663-27-1. Supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute.

[3]Hematology-Oncology Service, Department of Medicine, Wilford Hall US Air Force (USAF) Medical Center, Lackland Air Force Base, San Antonio, Tex.

[4]The voluntary, fully informed consent of each patient was obtained in accordance with AFR 160-9. This material reflects the personal views of the authors and does not represent policy or opinion of the USAF or the Department of Defense.

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TABLE 1.—Responses among 39 lung cancer patients treated with CACP

Histologic type	No. of patients*	Response		Ref No.
		PR	Other	
Adenocarcinoma	8 (5)	1	7	1,†
Large cell	5 (5)		5	†
Small cell	4 (4)		4	†
Undifferentiated	4	1	3	5
Anaplastic	3 (3)		3	†
Epidermoid	1		1	1
Alveolar cell	1		1	1
NOS‡	13	—	13	3,9
Totals	39	2	37	

*Nos. in parentheses refer to patients from this study.
†This study.
‡Histologic subtype not otherwise specified.

tions of CACP (1–5). Few series have subsequently intensively evaluated this agent against specific tumor types. CACP is active against testicular (6) and ovarian (7) cancer but is inactive in colon cancer (8).

In addition to the 17 patients with lung cancer whom we treated, an additional 22 patients have been gathered from other sources (1,3,5,9) and are summarized in table 1. Dosages and schedules of administered drug cannot be discerned accurately from these sources, but only one additional PR has been identified.

The dose-limiting toxic effects of CACP are nausea and vomiting in most patients. Tinnitus occurred in only one patient but no objective evidence of ototoxicity was detected by audiography.

Although a single long-term favorable effect was seen among our patients, we feel that this agent, when used alone in patients with lung cancer, does not appear to be of value in the dosage and schedule employed in this study.

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Phase II Study of VP-16-213 Versus Dianhydrogalactitol in Patients With Metastatic Malignant Melanoma[1,2,3]

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Disseminated malignant melanoma has been a most refractory neoplasm with both quantitative and qualitative antitumor response to most chemotherapeutic agents of limited value (1–3). This prompts continued search for new active agents in this most refractory neoplasm.

VP-16-213 is a new agent with limited trials in man. Data from preliminary studies indicate that this agent causes initial metaphase arrest followed by an effect which prevents cells from entering the mitotic phase of the cell cycle. The most pronounced effect of this agent was seen in the L1210 tumor model, but activity has also been seen in many of the animal sarcomata. Excretion of the drug occurs mainly through the biliary system (50%) and the kidney (30%).[5] A phase I and II study performed at this institution in patients with advanced breast cancer had indicated the tolerance dose for VP-16-213 to be 125 mg/m^2 every other day for three doses (4).

Dianhydrogalactitol is a diepoxide and apparently the active metabolite of dibromodulcitol. Its mechanism of action includes alkylation and inhibition of DNA synthesis among other possible mechanisms.[6] Antitumor activity was noted in the L1210 leukemia as well as B16 melanoma. Animal toxicology revealed myelosuppression to be the major side effect with gastrointestinal and hepatic toxicity also apparent. Our phase I trial indicated that a dose of 25–30 mg/m^2 on each of 5 successive days produced consistent but non-life-threatening toxicity.

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[2]Supported in part by contract CM-57044A from the National Institutes of Health, Department of Health, Education, and Welfare.

[3]VP-16-213: NSC-141540; 4'-demethylepipodophyllotoxin, 9-(4,6-*O*-ethylidene-β-D-glucopyranoside.

Dianhydrogalactitol: NSC-132313; CAS reg. No. 23261-20-3; galactitol, 1,2:5,6-dianhydro-.

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[5]Goldsmith M. Clinical brochure: 4-demethyl-epipodophyllotoxin-β-D-ethylidene glucoside (NSC-141540) VP-16-213. Prepared by the National Cancer Institute (NCI), Bethesda, Md, Jan 1973.

[6]Handelsman H. Clinical brochure: galactitol. Prepared by the NCI, Bethesda, Md, 1974.

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MATERIALS AND METHODS

Twenty-six patients with disseminated melanoma participated in this clinical trial. There were 16 men and ten women with a median and mean age of 56 years. Twenty-three of the 26 patients had visceral-dominant disease with multiple organ sites of tumor involvement. Only two of the 26 patients had previously been exposed to chemotherapy, one in each primary treatment group. Previous chemotherapy included methyl-CCNU and DTIC in one patient and methyl-CCNU and adriamycin in the other patient. Two patients had previously received immuno-adjuvant therapy utilizing bacillus Calmette-Guérin without effect. All of the patients had confirmation of the nature of their primary lesion and had maintained a reasonable state of nutrition with a performance score (Eastern Cooperative Oncology Group scale) of ≥ 3, but demonstrated progressive tumor growth prior to the study. All patients had wbc counts > 4100 cells/mm^3 and platelet counts $> 130{,}000$ platelets/mm^3. All of the patients had adequate renal function with creatinine levels < 1.5 mg/100 ml. An informed consent was obtained from all patients. Pretreatment assessment was as previously outlined (2). The patients were randomly allocated to either one of two treatment programs after stratification as to the presence or absence of visceral-dominant disease.

VP-16-213 was given as a direct iv infusion at a dose of 125 mg/m^2 every other day for three doses. Dianhydrogalactitol was given at a dose of 30 mg/m^2 on each of five successive days by rapid iv infusion in 18 of the patients; however, two others received 35 mg/m^2 and one patient received 25 mg/m^2. Of the patients receiving either primary or secondary therapy with VP-16-213, six had an escalation of dose by 10% and one had a decrease in dose by 25% on subsequent treatments because of the lack of toxicity. After dianhydrogalactitol therapy, only two patients had a dose adjustment, one decreased by 10% and the other by 20%. Subsequent reassessment was accomplished at 4 weeks after completion of chemotherapy and included a repetition of pretreatment studies. An objective response was defined as a decrease of $\geq 50\%$ in the product of the perpendic-

ular diameters of the indicator lesion(s) lasting a minimum of 4 weeks. Any patient whose disease had progressed after the initial chemotherapeutic effort was treated with the alternate program.

RESULTS

Table 1 indicates the relative lack of antitumor effect as reflected in the objective decrease in tumor size. Only one patient experienced an objective response, that being a complete response after dianhydrogalactitol therapy. Stability of disease, however, did follow, not uncommonly, the use of VP-16-213 with nine of 12 patients having stability of disease lasting from 8 to 13 weeks while two of 14 patients achieved stability of disease for a duration of 8 weeks after dianhydrogalactitol.

Toxicity observed with these agents is shown in table 2. Predictable but mild nausea and vomiting occurred. Alopecia more commonly followed the use of VP-16-213. Myelosuppression occurred after the use of each of the agents with the myelosuppression after VP-16-213 not associated with significant platelet suppression. After dianhydrogalactitol, however, platelets were depressed to $< 130{,}000/mm^3$ in 11 of 21 patients. The mean survival after primary therapy with VP-16-213 was 16 weeks (18-week median) while after primary therapy with dianhydrogalactitol the mean survival was 12 weeks (12-week median). Five patients are still alive in each of the treatment groups at the time of this report. After secondary therapy, survival was expectedly shorter with a 10-week mean survival after VP-16-213 (12-week median) and a 9-week mean survival after dianhydrogalactitol (7-week median). Five of these patients, however, do remain alive at this time.

Neither VP-16-213 nor dianhydrogalactitol was helpful in the treatment of this group of patients with disseminated malignant melanoma. Neither agent displayed appreciable antitumor effect, as reflected by an objective diminution in tumor size, with the exception of one patient who responded to dianhydrogalactitol and whose response continues at 26 weeks. However, patients did have periods of stability more commonly after VP-16-213 therapy. Since only two of these 26 patients had previously been exposed to cytotoxic chemotherapy, an adequate clinical trial occurred for these new agents and neither agent displays enough antitumor effect to prompt its continued use in patients with this disease at dose regimens used in this study.

TABLE 1.—Results of primary and secondary treatment of patients with VP-16-213 and dianhydrogalactitol

	No. of responses/ No. of patients treated	
Treatment	VP-16-213	Dianhydrogalactitol
Primary	0/12	1/14
Secondary	0/7	0/7
Total	0/19	1/21

TABLE 2.—Toxic effects after primary and secondary therapy

	No. of patients with toxic reaction	
Toxic effect	VP-16-213 (19 patients)	Dianhydrogalactitol (21 patients)
Nausea, vomiting	11	13
Diarrhea	1	1
Alopecia	8	3
Leukocytes $4100/mm^3$	8*	14†
Platelets $130{,}000/mm^3$	0	11‡

*Nadir, 2200 cells/mm^3 on Day 11 lasting 8 days.
†Nadir, 2500 cells/mm^3 on Day 17 lasting 18 days.
‡Nadir, 92,000 platelets/mm^3 on Day 21 lasting 9 days.

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Vinblastine, Procarbazine, and Cytosine Arabinoside in Combination for Reinduction of Childhood Acute Myelocytic Leukemia [1,2,3]

Brief Reports and Preliminary Communications

Norma K. C. Ramsay,* Peter F. Coccia,[4] William Krivit, Clara D. Bloomfield, and Mark E. Nesbit[5]

In an attempt to find a combination of chemotherapeutic agents capable of inducing a CR in children with acute myelocytic leukemia (AML) who had failed induction or relapsed on standard therapy, we combined three agents known to be markedly myelosuppressive but with different systemic toxicity. Two of the agents, vinblastine and procarbazine, have shown minimal activity as single agents in AML (1–3), but have been effective in the treatment of Hodgkin's disease and non-Hodgkin's lymphoma (4). The third agent, cytosine arabinoside (ara-C), is effective for the induction and maintenance of AML (5).

PATIENTS AND METHODS

Eight children with AML who had received prior intensive treatment, including ara-C, were studied. The morphologic diagnosis was based on criteria used at this institution (6). Three patients had failed induction therapy, four had relapsed after an initial CR, and one had relapsed after two previous CRs. All eight patients had previously received ara-C, and either failed induction or relapsed while on this drug. None of the patients had received prior vinblastine or procarbazine, however, four of eight had received combinations which included vincristine, a much less myelosuppressive vinca alkaloid. Details of prior therapy are outlined in table 1.

The treatment regimen consisted of vinblastine (5.0 mg/m^2 by iv push on Days 1, 8, and 15), procarbazine (100 mg/m^2 orally daily × 14 days), and ara-C (100 mg/m^2/day by iv push in divided doses every 8 hours on Days 1–5). The course of induction therapy was repeated on Day 28 if there was evidence of response but the marrow did not meet the criteria for CR. If a CR was obtained, the patient was placed on a maintenance regimen of monthly courses of vinblastine, procarbazine, and ara-C alternating with vinblastine and procarbazine alone, administered in doses identical to those in the induction regimen. Patients were taken off the study if they had progressive disease (PD).

All patients were evaluated before treatment with aspiration and trephine biopsies of the bone marrow, hemograms, uric acid determinations, and liver and renal function tests. Throughout induction, hemograms were repeated at least every other day and liver and renal function tests were performed at least twice a week. Bone marrow examinations were performed 7–14 days after completion of each course of therapy. Criteria for evaluation were those used by the Children's Cancer Study Group (7) with a CR defined as a normal marrow which contains < 5% blast cells, a normal hemogram, and a physical examination which does not indicate any evidence of residual disease. A PR is defined as a bone marrow containing 6%–25% blast cells with the peripheral blood containing > 500 granulocytes/mm^3 and < 5% blast cells.

RESULTS

Response to induction therapy is presented in table 1. Three children achieved a CR after one course of therapy and were placed on maintenance therapy. Two patients showed a PR after two courses. One of these patients refused further therapy and the other failed to show further improvement with a third course. Two patients showed no response (NR) after two courses of therapy and

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[3]Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate.

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride.

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride.

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TABLE 1.—Patient data

Patient No.	Sex/age at diagnosis	Diagnosis	Time since diagnosis (mos)	Previous therapy			Vinblastine, procarbazine, ara-C therapy		
				Drugs*	Induction attempts	CR	Induction courses	Response	Duration of maintained remission (mos)
1	F/14	AML	3	D,ara-C,6-TG,C,P	2	0	1	CR	11
2	M/12	AML	13	D,ara-C,5-aza, 6-TG, C, V	2	2	1	CR	4
3	M/14	AML	25	A,ara-C,6-TG, C, CCNU	2	1	1	CR	2
4	F/14	Erythroleukemia	16	D,ara-C,6-TG,P	1	1	2	PR	—
5	M/14	AML	3	D,ara-C,V,P, 5-aza	1	0	3	PR	—
6	M/10	AML	7	D,ara-C,6-TG, V,P,5-aza, L-asp, VP-16-213	4	1	2	NR	—
7	F/12	AML	8	D,ara-C,6-TG,C	2	1	2	NR	—
8	M/8	AML	3	D,ara-C,6-TG,C,V	2	0	1	NR	—

*D = daunorubicin; A = adriamycin; 6-TG = 6-thioguanine; C = cyclophosphamide; V = vincristine; P = prednisone; 5-aza = 5-azacytidine; L-asp = L-asparaginase.

were taken off the study. One patient had PD after one course of therapy and was removed from the study. Remission duration for the three patients who attained CR was 2–11 months (table 1).

Severe bone marrow suppression and peripheral pancytopenia occurred in all 13 courses of therapy evaluated. The nadir wbc count was < 1000 cells/mm^3 in five of eight patients while the nadir platelet count was < 25,000 platelets/mm^3 in all eight patients. The duration of severe granulocytopenia was 3–30 days with a median of 10 days and the duration of thrombocytopenia was 6–31 days. Four patients developed sepsis during induction and all responded to antibiotics and granulocyte transfusions. Nonhematologic toxicity was minimal and included nausea and vomiting in most patients during the first few days of therapy and stomatitis in one. There was no evidence of hepatic, renal, or neurologic toxicity. The only toxicity seen during maintenance therapy consisted of mild myelosuppression.

DISCUSSION

The induction of a complete bone marrow remission is believed necessary to prolong survival in AML (8). However, about 30% of the patients do not attain an initial remission with present-day therapy and most patients who do not attain a CR relapse within 1 year. It is evident that new combinations of agents are necessary both to induce remissions and to prolong remission duration if survival is to increase significantly in the future.

The development of severe bone marrow hypoplasia appears to be necessary to induce CR in AML (8). In the present study we describe our results with a myelosuppressive combination which was successful in inducing remission both in patients who failed induction regimens with more conventional agents and in patients who relapsed while on maintenance therapy. All eight patients in this study had previously received ara-C; three had failed previous myelosuppressive induction regimens which included ara-C and five had relapsed while receiving maintenance therapy which included ara-C as one of the agents. The fact that three of the eight patients attained a CR when vinblastine and procarbazine were combined with ara-C demonstrates that this combination is effective in reinducing remission in recurrent childhood AML, even in patients unresponsive to ara-C given in other combinations. All three patients who attained a CR did so after one course of therapy. Administration of one or two more courses in the remaining five patients did not alter their bone marrow status.

Three of eight patients (37.5%) studied attained a CR and five of eight (62.5%) responded with this combination of agents. Therapy was relatively nontoxic aside from the expected severe myelosuppression. In view of these preliminary results, further

studies of this combination, both for remission induction and for maintenance of remission in childhood AML, appear warranted.

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Adriamycin and CCNU in the Treatment of Advanced Breast Cancer[1,2,3]

Brief Reports and Preliminary Communications

H. B. Muss,* D. R. White, F. Richards II, M. R. Cooper, and C. L. Spurr[4,5]

Adriamycin has been used extensively in the treatment of patients with advanced breast cancer and has been shown to produce response rates of 30%–40% even in patients previously treated with intensive chemotherapy (1). When used as a single agent the response to adriamycin is usually of short duration with a median response of 4 months (2). The nitrosoureas have shown activity in patients who have had prior exposure to alkylating agents and may be considered as therapeutic alternatives in breast cancer patients who have received prior combination chemotherapy.

Bonnet et al (3) using BCNU obtained a 36% response rate (13 of 36 patients) in patients with advanced breast cancer. An earlier study of Ahmann et al (4), however, was discouraging in that no responses were seen in 16 patients treated with this agent. Clinical studies with CCNU in advanced breast cancer have shown response rates of 10% (two of 21 patients) (5) and 19% (seven of 36 patients) (6). The response rates to methyl-CCNU have been disappointing with only a 4% response rate (four of 100 patients) noted (7). Streptozotocin apparently has had little trial in this disease (8) and further data are needed to determine its role in breast cancer. It was anticipated that the addition of a nitrosourea to adriamycin would not only enhance the response rate but also increase the duration of response over that observed with adriamycin alone.

In an earlier study utilizing CCNU and adriamycin there were eight responses (two CRs + six PRs) in 13 patients (62%) (9). Another regimen using a combination of methyl-CCNU and adriamycin produced four responses in 22 patients (18%) (one CR + three PRs) (10). The present study was designed to evaluate the therapeutic benefit of the combination of CCNU and adriamycin in patients with advanced breast cancer refractory to a conventional five-drug regimen.

MATERIALS AND METHODS

Thirty-one patients with advanced breast cancer (all with histologically confirmed diagnosis) were consecutively placed on a protocol using CCNU and adriamycin. Twenty-seven patients had their performance status documented: 18 were bedridden < 50% of the time, eight were bedridden > 50% of the time, and one was completely bedridden. Twenty-four of the patients had had prior hormonal therapy: ablative in four, additive in 11, and additive and ablative in nine. No patient had been withdrawn from hormones within 3 months prior to entrance in the study. There was no difference in prior hormonal therapy in the three groups of patients. All patients had been previously treated with intensive five-drug combination chemotherapy using cyclophosphamide, vincristine, methotrexate, 5-fluorouracil, and prednisone. Patients who had unequivocal progression of disease on this combination were placed in the present study after drug toxicity had cleared. Patients received CCNU at a dose of 50 mg/m^2 orally on Day 1 and this was repeated every 4 weeks. Adriamycin was administered at a dose of 30 mg/m^2 iv on Day 1, then repeated every 2 weeks. A course was defined as one dose of CCNU on Day 1 and adriamycin on Days 1 and 14. Dosages were adjusted to maintain the wbc count > 3500 cells/mm^3 and the platelet count > 75,000 platelets/mm^3 before each dose of chemotherapy.

All patients had measurable disease. Clinical or radiographic measurements were used. Serial liver scans were used as indicators of clinical response. No patients had bone metastases only. Bone scanning was performed on all patients. In patients with a positive bone scan, conventional roentgenograms

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[2]Supported in part by Public Health Service grants CA-08037 and CA-12197 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Adriamycin: NSC-123127; CAS reg. No. 23214-92-8.

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-. Supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI.

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[5]We wish to thank Tanya Jackson and Anita Shore for their statistical help and Sue Elliott and Peggy Reece for their help in the preparation of this manuscript.

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TABLE 1.—Clinical characteristics and response to therapy

	Response			
	CR + PR	Stable disease	Progressive disease	Totals
No. of patients with—				
Response to CCNU/adriamycin	9/31 (29%)	10/31 (32%)	12/31 (39%)	31 (100%)
Response to prior chemotherapy	5/9 (56%)	5/10 (50%)	4/12 (33%)	14/31 (45%)
DFI < 2 yrs	3/9 (33%)	5/10 (50%)	5/12 (42%)	13/31 (42%)
Prior hormonal therapy	8/9 (89%)	8/10 (80%)	8/12 (67%)	24/31 (77%)
Metastatic sites (CCNU/adriamycin)				
Skin/subcutaneous	7/9 (78%)	5/10 (50%)	5/12 (42%)	17/31 (55%)
Lymph node	4 (44%)	4 (40%)	4 (33%)	12 (39%)
Pleura	1 (11%)	2 (20%)	3 (33%)	7 (23%)
Bone	6 (67%)	7 (70%)	11 (92%)	24 (77%)
Lung	3 (33%)	2 (20%)	6 (50%)	11 (35%)
Liver	0 (0)	0 (0)	4 (33%)	4 (13%)
Brain	1 (11%)	1 (10%)	2 (17%)	4 (13%)
Age (median/mean)	59/57	55/53	56/57	
Median duration of response (range)	3.6 mos (1–12+)			

of positive areas were obtained. In no patient did pleural effusion constitute the only measurable disease.

A CR was defined as the complete disappearance of all observable and measurable tumor. A PR was defined as a 50% reduction in the product of the largest diameter of the tumor and its perpendicular. In partial responders who had bone metastases in addition to clinically measurable lesions, there could be no progression of bony disease either symptomatically, radiographically, or isotopically. All favorable responses had to last ≥ 1 month. Stable disease was defined as < 50% reduction in tumor size of measurable lesions without the appearance of new lesions (including bone scan) or clinical deterioration not due to drug toxicity.

RESULTS

The clinical characteristics and results of therapy of the 31 patients in this study are presented in table 1. One hundred and fifty-six courses of CCNU and adriamycin were administered for both an average and median of 5.0 courses per patient. Three patients had only one course before disease progression but are considered evaluable. Seventy-three percent of the calculated CCNU dosage and 63% of the adriamycin dosage were administered. Patients without bone metastases received 66% of the adriamycin dose and 79% of the CCNU. Those with bone metastases received 62% of the adriamycin dose and 71% of the CCNU. There was no significant difference between these two groups.

There were two CRs, both in patients who had extensive cutaneous and subcutaneous metastases only. Seven PRs were observed. Six had bone lesions which did not change clinically or radiographically. One of these patients had brain metastases that had been treated with prior whole-brain irradiation. All of these patients showed a > 50% decrease in all other measurable disease. The overall response rate was 29% with a median duration of response of 3.6 months and a range of 1–12+ months. There was no difference in response rates with respect to response to prior therapy, disease-free interval (DFI), or age (table 1). Distribution of metastases did not influence the response rate with the notable exception of liver metastases; patients with liver metastases did not respond or stabilize on the present program.

Toxic Effects

There was one death due to sepsis in a patient who was critically ill before treatment and who developed profound neutropenia within 2 weeks after the administration of her first dose of medication. Hematologic toxicity is presented in table 2. No patient developed a platelet count < 20,000 platelets/mm^3 and there were no bleeding complications.

TABLE 2.—Hematologic toxicity (nadir)

	No. of patients	Median duration* (days)	Range* (days)
Leukopenia (cells/mm³)			
< 1000	2	33	14–52
1000–2000	6	35	28–51
2000–3500	15	34	22–50
Thrombocytopenia (platelets/mm³)			
20,000–50,000	2	33	14–52
50,000–100,000	9	38	22–70

*Until recovery to wbc count > 3500 cells/mm³ and platelet count > 100,000 platelets/mm³.

No cardiac toxicity was observed. Nausea and vomiting, alopecia, and gastrointestinal distress occurred in almost all patients.

DISCUSSION

This study demonstrates that combination chemotherapy using adriamycin and CCNU is capable of producing objective responses in patients with advanced metastatic breast cancer refractory to the five-drug combination of cyclophosphamide, vincristine, methotrexate, 5-fluorouracil, and prednisone. The rate and duration of response, however, appear to be very similar to that produced by adriamycin when used as a single agent. Prior regimens have shown that adriamycin administered every 2 weeks in conjunction with other agents is as effective as when given every 3 weeks in patients with breast cancer (11). Previous experience has demonstrated that with appropriate dosage modification for marrow toxicity, CCNU is well tolerated and efficacious when administered every 4 weeks (12). No synergistic or additive effect of CCNU and adriamycin was demonstrated with the schedule used in this study. It is not anticipated that increasing the dosages with an almost certain increase in the considerable bone marrow toxicity observed would enhance the value of this combination. The combination of CCNU and adriamycin appears to be of limited value in the therapy of patients with advanced breast cancer who have been exposed to intense prior chemotherapy. Its use in patients with untreated advanced recurrent cancer, especially in those patients with extensive local disease recurrence, needs to be further explored.

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Pulmonary Toxicity in Long-Term Administration of BCNU[1,2]

Brief Reports and Preliminary Communications

Paul Y. Holoye,[3,*] Daniel E. Jenkins,[4] and S. Donald Greenberg[4]

Many chemotherapeutic agents have delayed and unexpected toxicity. Until the appearance of bleomycin, pulmonary toxicity was unusual and limited to the alkylating agent busulfan (1,2) and, occasionally, to methotrexate (3). Since 1964 the nitrosoureas have been introduced in clinical practice and have shown therapeutic effectiveness against lymphoma, leukemia, brain tumor, gastrointestinal cancer, bronchogenic carcinoma, melanoma, multiple myeloma, and Ewing's sarcoma (4–8). Chronic toxicity has not been described except for a limiting cumulative bone marrow toxicity. We wish to report a case of interstitial pulmonary fibrosis developing after 3 years of low-dose BCNU administered for brain tumor.

CASE REPORT

A 19-year-old white man developed progressive headache, nausea and vomiting, intermittent diplopia, and cervical neck pain. A grade I astrocytoma was partially removed from the right cerebellum on April 13, 1971. The patient was referred to the M. D. Anderson Hospital and Tumor Institute where nystagmus and uncoordination of the right extremities were noted. He was started on Dilantin, phenobarbital, and prednisone at a dose of 80 mg/day. Five thousand rads of cobalt 60 were given in 7 weeks to the posterior fossa and the upper cervical spine between June 3 and July 20, 1971. In November 1971, he had persistent right cerebellar signs. BCNU (100 mg/m^2 iv) was given every 3 or 4 weeks. In 1972, the patient was able to walk, ride a bike, and resume full-time employment. The last course of BCNU was given in October 1974. The overall cumulative dose was 3330 mg or 2081 mg/m^2 given over 19 courses. Beginning in August 1974, the patient developed a dry, nonproductive cough associated with dyspnea on exertion. Bronchodilators and hyposensitization were tried. In March 1975, spirometry and blood gases were abnormal (PO_2 45, PCO_2 16, FVC 1.1 liter, FEV_1 0.7). Antibiotics and intermittent positive pressure breathing gave temporary relief. He was re-admitted on April 3, 1975, to his local hospital where prednisone was increased from 30 to 50 mg/day prior to transferral to the Methodist Hospital, Houston, Texas. The initial physical examination revealed a blood pressure of 116/78, a respiration rate of 24/minute, a regular pulse rate of 134/minute, a marked retraction of the lower rib cage on inspiration, and bilateral inspiratory rales on both lung bases; no evidence of heart failure was found. The cbc, SMA-100, serum electrolytes, and urinalysis were normal. Chest roentgenograms showed a 10%–15% pneumothorax on the right side and a chronic interstitial change in both bases with bilateral fractured ribs (fig 1). Bronchoscopic examination was normal. Blood gases and spirometry revealed a combined obstructive and restrictive defect. A PO_2 of 78 mm Hg was maintained by an inspired oxygen concentration of 28%. On April 16, 1975, transbronchial lung biopsy led to bilateral pneumothorax managed by anterior chest tubes. A left open-lung biopsy was performed on May 13, 1975. The chest tubes were removed on May 18, 1975. On May 23, 1975, the patient was noted to be anxious. The next day, he was found without respiratory or cardiac function and resuscitation was unsuccessful; an autopsy was done within 45 minutes.

PATHOLOGIC FINDINGS OF THE LUNGS

Gross.—Both of the lungs were small, contracted, and bounded by fibrous thickening of the visceral pleura. The cut surfaces revealed a patchy distribution of interstitial fibrosis which tended to be most marked in the lower lobes. No solidification, thromboemboli, or infarcts were seen. The major bronchi were patent and showed no change. The pulmonary arteries and veins were unremarkable. A represent-

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[2]BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

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FIGURE 1.—Roentgenogram of the chest showing bilateral pulmonary fibrosis and a small right pneumothorax.

ative portion of each lower lobe was submitted to the Diagnostic Microbiology Laboratory for routine culture for bacteria, fungi, and mycobacteria. The microbiologic cultures were all negative.

Microscopic.—Multiple-tissue preparations from both lungs revealed a similar pattern. The interstitium contained abundant fibrous connective tissue which was most prominent within the alveolar septa. The organized interstitial fibrosis was patchy and most severe in preparations from the lower lobes (fig 2) and surrounded occasional bronchioles and small muscular pulmonary arteries. There was no inflammatory reaction. Hyperplasia of the type II alveolar epithelial lining cells was seen as a cuboidal metaplasia. The bronchi were unremarkable. No thromboemboli were seen. Medial hypertrophy of the small muscular pulmonary arteries was noted. The visceral pleura was broadened by extensive proliferation of fibrous connective tissue. No birefringent or foreign material was seen.

Comment.—The gross and microscopic findings were those of a "bland" nonspecific interstitial pul-

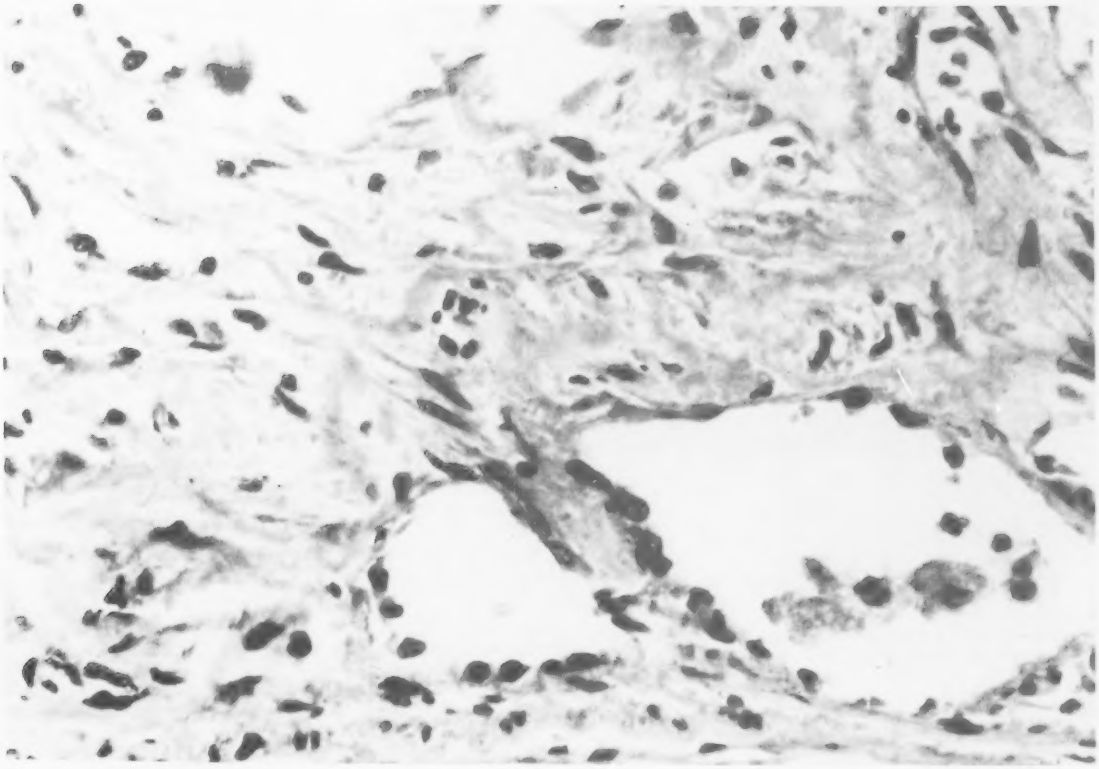

FIGURE 2.—Photomicrograph of lung showing the bland nonspecific interstitial pulmonary fibrosis (H & E stain, × 250).

monary fibrosis involving alveolar septa, peribronchiolar, and pleural tissue. There was no inflammatory reaction.

Associated pathologic findings.—Significant associated pathologic findings were right ventricular hypertrophy with dilatation (cor pulmonale) and residual astrocytoma, grades I–II, of the right cerebellar hemisphere with invasion of the pons and brain stem.

DISCUSSION

The appearance of dyspnea and pulmonary infiltrate in a patient treated with corticosteroids and chemotherapy suggests the diagnosis of opportunistic infection. However, at autopsy which was performed immediately after death, multiple microscopic tissue preparations from all five lobes of the lungs revealed only a "bland" nonspecific interstitial fibrosis. There was no inflammatory reaction and cultures of representative portions of both lower lobes were negative for bacteria, fungi, and mycobacteria. During his last 3 years of life, the patient worked in a tree nursery where he was exposed to a large variety of organic dusts. However, pathologic findings are not diagnostic of allergic alveolitis. We conclude therefore, by exclusion, that the interstitial fibrosis was iatrogenic and related to the use of BCNU (9,10).

Few references are available concerning the possible pulmonary toxicity to these agents. In preliminary clinical trials, 1-methyl-3-nitro-1-nitrosoguanidine caused pulmonary vascular damage (11). Iriarte et al (12) treated 14 children with BCNU (150 mg/m^2) for 3 consecutive days; four children developed bilateral pulmonary infiltrate 2–4 months later.

The initial animal data did suggest the possibility of a pulmonary toxicity. 1-Methyl-3-nitro-1-nitrosoguanidine was evaluated in dogs and rhesus monkeys. In the latter species, the drug induced pulmonary edema, pleural effusion, and acute respiratory

distress at doses of 20–40 mg/kg. In the dog, pulmonary edema without pleural effusion was noted.[5] BCNU appeared as a more promising agent because of its increased activity against animal tumor systems compared with 1-methyl-3-nitro-1-nitrosoguanidine. However, it retained the capability of inducing acute pulmonary toxicity in the rhesus monkey and dog (13). Very few animal data are pertinent to the situation of our patient, ie, the use of small nonmyelosuppressive doses repeated over a long period of time.

The pathogenesis of the interstitial fibrosis is not evident. One possible explanation would be a local effect by an exhaled metabolite of BCNU. This toxicity is probably rare because few patients have the opportunity to receive the drug over such a prolonged period of time. However, adjuvant chemotherapy with various nitrosourea compounds is being introduced against bronchogenic carcinoma and this complication might become more frequent.

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ANNOUNCEMENTS

American Board of Internal Medicine Certifying Examination in the Subspecialty of Medical Oncology

October 18, 1977

The American Board of Internal Medicine will administer the Certifying Examination in the Subspecialty of Medical Oncology on Tuesday, October 18, 1977. Application forms may be requested beginning January 1, 1977, and must be returned by March 15, 1977.

Requests for information and application forms should be directed in writing to: Registration Department, American Board of Internal Medicine, 3930 Chestnut St, Philadelphia, Pa 19104.

Availability of Interferon

In order to foster increased research on interferon in relation to cancer, the National Cancer Institute, National Institutes of Health, Bethesda, Md, is in the process of obtaining a supply of human leukocyte, human fibroblast (diploid) cell, and human lymphoblastoid cell interferons. This material will be made available to qualified scientists who have obtained support for their research but are in need of interferon. The human leukocyte and fibroblast interferons are being prepared in a manner which will make them usable for clinical studies.

Scientists interested in obtaining some of this material should submit their requests to the Interferon Working Group, NCI, NIH, which will be responsible for the distribution of this material. The request should specify the type and amount of interferon desired and should include a complete description of the study for which the interferon is needed and the source of support. If it is to be used in a clinical study, the clinical protocol should also be included.

It should be stressed that limited amounts are available and only the most scientifically deserving proposals will be considered. Therefore, it is to the applicant's advantage to be complete.

Requests should be addressed to: Chairman, Interferon Working Group, NCI, NIH, Bg 31, Rm 3A03, Bethesda, Md 20014.

Symposium on "Use of Athymic (Nude) Mice in Cancer Research"

Columbus, Ohio, June 15–16, 1977

The Battelle Memorial Institute (Columbus, Ohio) and the Division of Cancer Treatment of the National Cancer Institute (Bethesda, Md) announce a symposium on "Use of Athymic (Nude) Mice in Cancer Research" (Drs. David Houchens and Artemio Ovejera, co-chairmen) to be held at Battelle Memorial Institute, 505 King Ave, Columbus, Ohio, on June 15–16, 1977.

This symposium will cover topics of mouse development, biology of nude mice, immunologic studies, tumor development, and treatment of tumors.

For further information and registration forms write to: Ms. Susan Armstrong, BATTELLE, Columbus Laboratories, 505 King Ave, Columbus, Ohio 43201.

Medical Oncology Practice Study

A study of the professional activities of Medical Oncology is currently being implemented by the Medical Activities and Manpower Projects, Division of Research in Medical Education, University of Southern California School of Medicine. The study is being conducted in conjunction with the American Society of Clinical Oncology during the week of March 25 through April 2, 1977. Since it will provide our specialty with information of value for educational and manpower planning, we strongly urge those of you selected to participate in the study to cooperate fully.

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